Oxido-Reduction States and Natural Homologue of Ubiquinone (Coenzyme Q) in Submitochondrial Particles From Etiolated Mung Bean (Phaseolus aureus) Seedlings

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Abstract. A procedure for the isolation of submitochondrial particles in quantity from etiolated Mung bean (Phaseolus aureus) seedlings is described. Using a combination of acetone extraction and 2 systems of thin layer chromatography ubiquinone has been isolated. The isolated ubiquinone migrates coincident with authentic ubiquinone-10 in reversed phase thin layer partition chromatography, gives a positive Craven's test, and has oxidized and reduced spectra characteristic of ubiquinone. The quinone is partially reduced under steady-state electron transfer conditions with both succinate and NADH as substrates and is almost completely reduced under anaerobic conditions with either substrate. The concentration of ubiquinone in the particle is of the order of 4.4 mmole per mg particle protein, approximately equal to that found in similar submitochondrial particles from beef heart. It is tentatively concluded that ubiquinone-10 is a functional member of the mitochondrial electron transfer chain of Phaseolus aureus.

Although the functional role of ubiquinone as an electron transfer component of the mitochondrial electron transfer chain has been well established in animal systems (for recent reviews see reference 1) its status in mitochondrial systems of higher plants has received scant attention. It is the purpose of this communication to report the steady-state oxido-reduction levels of UQ in submitochondrial particles isolated from Mung bean seedlings during several functional states as well as to report the molecular species of UQ native to the mitochondrial electron transfer chain of this organism. This has been the subject of 2 recent reports in abstract form (2, 3).

Materials and Methods

Plant Culture. For the large scale preparation of mitochondria 1.4 kg of Mung beans (Phaseolus aureus, var. Jumbo) were washed thoroughly in tap water, soaked overnight and placed in a 10 gallon, light-tight plastic container perforated at the bottom. The preparation was washed thrice daily with tepid tap water and harvested at the sixth or seventh day, yielding approximately 14 kg of etiolated Mung bean seedlings.

Large Scale Preparation of Mitochondria. The preparation of Mung bean seedlings was washed thoroughly with deionized water (3 rinses), cooled, and homogenized for 3 seconds at full speed in a commercial sized Waring Blender. Homogenization was performed in a cold solution (solution A) containing 0.3 M sucrose, 1 M EDTA, 0.1% bovine serum albumin fraction V (BSA), 0.05% cysteine HCl, and 10 M N₃HPO₄, the pH of which had been adjusted to 7.8 immediately prior to use. Approximately equal volumes of tissue and solution were present during homogenization. The resulting suspension, at pH 6.9 to 7.1, was filtered through 12 layers of cheesecloth, and the cloudy filtrate subjected to 48,200g in the Szent-Gyorgyi and Blum continuous flow Sorvall SS-34 rotor. The flow rate was maintained at 150 ml per minute with the Sorvall RC-2B centrifuge chamber set at 0 to 2. The resulting pellet was suspended in a cold solution containing 0.3 M sucrose, 0.1% BSA (fraction V), 0.1 M EDTA, 1 M ATP, 5 M MgSO₄, and 1 M sodium succinate, pH 7.5, to a concentration of 30 mg protein per ml. The ability of the mitochondrial preparation to oxidize succinate and NADH, and the degree of respiratory control by ADP, was assayed at this stage in the preparation to ensure the viability of the mitochondria.

Preparation of Submitochondrial Particles. The procedure employed for the preparation of submitochondrial particles from Mung bean mitochondria was similar to that described by Beyer (4) for beef heart electron transfer particles. The mitochondrial
suspension in solution C was treated with sonic vibrations with a Branson Sonifier, model S-75, 20 kc, for 30 seconds at a power output of between 6 and 7 amperes. The volume of the suspension undergoing sonic treatment was 20 ml and was maintained below 6° during sonic treatment by employing a glass jacketed 30 ml beaker around which was circulated water at 0°. Following sonic treatment the suspension was adjusted to pH 7.5 with 1 N KOH and centrifuged at 16,000g for 10 minutes. The cloudy supernatant solution was decanted so as to exclude a fluffy layer covering the hard packed pellet and centrifuged at 150,000g (Beckman model L ultracentrifuge, number 50 rotor) for 30 minutes. The clear, slightly pink supernatant, containing Mung bean cytochrome c (J. Kuner, unpublished observation) was saved for cytochrome c isolation. The pellet, containing Mung bean electron transfer particles (MB-ETP), was suspended in solution C by homogenization in a close fitting Teflon-glass homogenizer and centrifuged again at 150,000g for 30 minutes. The pellet was suspended in solution C to a protein concentration of 30 mg per ml. A flow diagram for the preparation of MB-ETP is shown in Scheme 1. A typical yield at the R4 stage was 925 mg protein and 276 mg protein at the R7 stage.

**Analytical Methods.** The redox states and quantities of UQ in MB-ETP were assayed according to the extraction method of Pumphrey and Redfearn (5) as described by Redfearn (6) using either FeCl₂ or AuCl₃ as oxidant. The quantity of UQ extracted from MB-ETP was determined by obtaining the difference spectrum, in a Cary model 15 spectrophotometer, of oxidized minus reduced samples between 320 and 240 nm and determining the difference at 275 nm, or by noting the loss of absorbancy of the sample at 275 nm upon reduction with NaBH₄ in a Beckman model DU-2 spectrophotometer. The concentration of UQ was calculated from the molar extinction coefficient ($\epsilon_{\text{ex}} - \epsilon_{\text{red}}$) 275 = 12,250 (7).

UQ was isolated from MB-ETP for characterization of the molecular species, i.e., the number of isoprenoid groups in the long aliphatic side chain at position 6 on the quinone nucleus, by the following procedure. Four ml of a suspension of MB-ETP at a protein concentration of 30 mg per ml were extracted at 25° with 40 ml of acetone and the suspension was centrifuged at 27,000g for 20 minutes. The clear yellow supernatant solution was decanted and the pellet was reextracted with 20 ml of acetone, centrifugation repeated, the 2 extracts combined, and evaporated to dryness in the dark under a stream of nitrogen. The extract was dissolved in 5 ml of acetone and applied as a band to a Gelman type SA thin layer chromatographic sheet adjacent to a spot of authentic UQ-10. The chromatogram was developed in a Gelman Thin Layer Chromatography chamber with a solvent system consisting of 3 volumes of benzene to 1 volume of chloroform. Although this system does not adequately separate the various homologues of UQ it does lead to some purification from phospholipids and unknown compounds of yellow color. The band which migrated in the region of authentic UQ-10 was excised, cut into small pieces and extracted with acetone. The extract was used for 1 of 3 purposes. When either UV spectrophotometry or a modified Craven's test for UQ was to be performed, the chromatographed extract was taken to dryness under a stream of nitrogen and dissolved in redistilled ethanol. The UV spectrum of oxidized and reduced UQ was obtained using a Unicam SP-800A recording spectrophotometer. The quinone was reduced by adding several grains of NaBH₄. A modified Craven's test for UQ was performed according to the description of Page et al. (8). The blue color which developed in the presence of the purified extract was compared with that obtained with authentic UQ-10 by obtaining spectra between 700 and 500 nm.

For the chromatographic identification of the Mung bean UQ homologue the reversed-phase partition chromatographic system of Wagner et al. (9) was employed except that paraffin impregnated Gelman type SA thin layer chromatographic sheets were used.

Protein was determined by a biuret procedure (10) after solubilization of MB-ETP with deoxycholate. Rates of oxygen consumption were determined with a Clark electrode in a 3 ml plexiglass chamber at 25°C. Signals from the Clark electrode were monitored with a Beckman DU-2 spectrophotometer.
electrode were monitored as described by Estabrook (11) using a Sargent SR recorder. Dissolved oxygen was assumed to be 240 \( \mu \text{M} \) at 25\(^\circ\)C. All assays of oxygen consumption were determined in a medium consisting of 0.3 \( \text{M} \) mannitol, 5 \( \text{mM} \) MgCl\(_2\), 10 \( \text{mM} \) KCl, and 5 \( \text{mM} \) tris-phosphate, pH 7.5.

All chemicals were of reagent or spectral grade. UQ homologues containing 4, 6, 7, and 9 isoprenoid units were a generous gift of Dr. O. Wiss of Hoffmann-La Roche and Company, Basle, Switzerland, and UQ-10 was generously supplied by Dr. Arthur F. Wagner of Merk, Sharpe, and Dohme Research Laboratories, Rahway, New Jersey. Type III horse heart cytochrome \( c \) was purchased from Sigma Chemical Company, and NADH and ADP were obtained from PL-Biochemicals, Incorporated. Bovine serum albumin fraction V was purchased from Nutritional Biochemicals Corporation. Cleveland, Ohio.

Results

Respiratory Rates. Rates of electron transfer from NADH or succinate to molecular oxygen at 2 stages in the preparation of MB-ETP are shown in table I. At the mitochondrial stage (R4 in Scheme 1) the oxidation of both NADH and succinate was stimulated by cytochrome \( c \) and ADP. Stimulation by ADP was taken as an indication that some degree of respiratory control was retained by this type of preparation despite the rather vigorous treatment employed in an attempt to obtain large quantities of particles instead of a qualitatively superior mitochondrial preparation. Ikuma and Bonner (12) have shown that Mung bean mitochondria (MB-Mw), prepared with the intention of high quality, but yielding small amounts of MB-Mw, are capable of high respiratory control with succinate and NADH as substrates. The preparation of Ikuma and Bonner (12) oxidizes succinate at a greater rate than the mitochondrial preparation reported herein.

MB-ETP oxidized NADH at rates greater than MB-Mw in the presence of cytochrome \( c \) (table I) by a factor of approximately 1.6. It should be noted that characteristic of this type of preparation is the lack of response to ADP. Contrary to an increase in the rate of NADH oxidation catalyzed by MB-ETP, this particulate preparation oxidized succinate at a rate less than the parent mitochondrial preparation (table I), indicating either loss or damage of the succinate to UQ segment of the succinic oxidase pathway in some of the particles. It might be noted that a similar type of submitochondrial particle (4) isolated from beef heart mitochondria, ETP (Mg\(^{2+}\)), assayed under conditions identical to that described in table I, catalyzed the oxidation of succinate in the presence of cytochrome \( c \) at a rate of 213 \( \mu \text{atoms} \) per minute per mg protein and NADH at a rate of 470 \( \mu \text{atoms} \) per minute per mg protein.

Steady State Redox Levels and Total Concentration of UQ. During electron transfer with either succinate or NADH as substrate, a portion of the UQ native to the MB-ETP was in the reduced state whereas UQ was almost totally in the oxidized state in the absence of reducing substrate (table II). NADH appeared to be a more effective reductant of UQ than succinate, a finding consistent with the rate limiting step of succinate oxidation in such MB-ETP being on the substrate side of UQ in the sequence of components. The difference between the redox state of UQ with the 2 substrates was not as apparent with the beef heart ETPH(Mg\(^{2+}\)) (table II) in which succinate oxidation was inhibited to a lesser extent relative to the NADH oxidase activity. NADH reduced UQ equally in the 2 types of submitochondrial particles reported in table II. The UQ of these well washed particles was almost totally in the oxidized state at the beginning of the experiments as indicated by the very low order of reduction in the absence of substrate. During substrate induced anaerobiosis, with either substrate, approximately 85\% of the UQ was reduced. Be-

<table>
<thead>
<tr>
<th>Particle type</th>
<th>Substrate</th>
<th>Additions</th>
<th>Oxygen consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB-Mw</td>
<td>Succinate</td>
<td></td>
<td>76</td>
</tr>
<tr>
<td>MB-Mw</td>
<td>Succinate</td>
<td></td>
<td>132</td>
</tr>
<tr>
<td>MB-Mw</td>
<td>Succinate</td>
<td>cyt. ( c )</td>
<td>157</td>
</tr>
<tr>
<td>MB-Mw</td>
<td>NADH</td>
<td></td>
<td>86</td>
</tr>
<tr>
<td>MB-Mw</td>
<td>NADH</td>
<td>cyt. ( c )</td>
<td>173</td>
</tr>
<tr>
<td>MB-Mw</td>
<td>NADH</td>
<td>cyt. ( c ) + ADP</td>
<td>215</td>
</tr>
<tr>
<td>MB-ETP</td>
<td>Succinate</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>MB-ETP</td>
<td>Succinate</td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>MB-ETP</td>
<td>Succinate</td>
<td>cyt. ( c ) + ADP</td>
<td>75</td>
</tr>
<tr>
<td>MB-ETP</td>
<td>NADH</td>
<td></td>
<td>136</td>
</tr>
<tr>
<td>MB-ETP</td>
<td>NADH</td>
<td>cyt. ( c )</td>
<td>272</td>
</tr>
<tr>
<td>MB-ETP</td>
<td>NADH</td>
<td>cyt. ( c ) + ADP</td>
<td>272</td>
</tr>
</tbody>
</table>

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Table II. UQ Concentration and Redox Levels in ETP During Electron Transfer and Anaerobiosis

Reduced levels of UQ during electron transfer were determined by adding substrate during rapid stirring and stopping the reaction within 5 to 10 seconds. Reduced levels of UQ at anaerobiosis were determined by adding substrate, mixing the contents of the assay, flushing the gas phase with nitrogen, capping, and incubating for 10 minutes to ensure oxygen depletion. In some instances a few grains of solid Na₂S₂O₄ were added at the end of the incubation period to ensure complete reduction. Each assay contained, in a volume of 1.1 ml: ETP, 30 mg protein; 300 μmoles mannitol; 5 μmoles MgSO₄; 10 μmoles KCl; 5 μmoles K₂HPO₄, pH 7.5; and either 50 μmoles succinate or 2.5 μmoles NADH. Other analytical details are described in the Methods section. ETPH(Mg²⁺) refers to beef heart electron transfer particles (4).

<table>
<thead>
<tr>
<th>Particle</th>
<th>Substrate</th>
<th>Total UQ</th>
<th>Steady state</th>
<th>Anaerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μmoles per mg protein</td>
<td>UQ, % reduced</td>
<td></td>
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<tr>
<td>MB-ETP</td>
<td>None</td>
<td>4.37¹</td>
<td>...</td>
<td>6</td>
</tr>
<tr>
<td>MB-ETP</td>
<td>Succinate</td>
<td>38</td>
<td>...</td>
<td>88</td>
</tr>
<tr>
<td>MB-ETP</td>
<td>Succinate + S₂O₄²⁻</td>
<td>92</td>
<td>...</td>
<td>93</td>
</tr>
<tr>
<td>MB-ETP</td>
<td>NADH</td>
<td>56</td>
<td>...</td>
<td>84</td>
</tr>
<tr>
<td>MB-ETP</td>
<td>NADH + S₂O₄²⁻</td>
<td>93</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ETPH(Mg²⁺)</td>
<td>None</td>
<td>4.22¹</td>
<td>...</td>
<td>5</td>
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<tr>
<td>ETPH(Mg²⁺)</td>
<td>Succinate</td>
<td>49</td>
<td>...</td>
<td>77</td>
</tr>
<tr>
<td>ETPH(Mg²⁺)</td>
<td>Succinate + S₂O₄²⁻</td>
<td>85</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ETPH(Mg²⁺)</td>
<td>NADH</td>
<td>55</td>
<td>...</td>
<td>72</td>
</tr>
<tr>
<td>ETPH(Mg²⁺)</td>
<td>NADH + S₂O₄²⁻</td>
<td>87</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

¹ Values refer to the mean values of 16 preparations of MB-ETP and 7 preparations of the beef heart ETPH(Mg²⁺).

Fig. 1. Reversed-phase thin layer partition chromatograph of UQ homologues and MB-ETP extract. Conditions are described in the Methods section. The developed chromatogram was photographed through a Kodak #47 gelatin filter without preliminary staining. The front seen above the line labeled “front” is the limit of paraffin infiltration of the supporting layer.
tween 4 and 9% additional reduction was observed with substrate plus dithionite, indicating that of the reducible UQ contained in MB-ETP a very large proportion was able to interact with the electron transfer chain. Only about 8% of the UQ was not reducible under these conditions suggesting a high degree of intactness of the electron transfer chain. Somewhat less of the total UQ was reduced under conditions of substrate plus dithionite in the beef heart system (table II).

MB-ETP contained approximately 4.4 mmoles of UQ per mg protein, a concentration approximately equal to that of the beef heart submitochondrial system (table II). These values are slightly lower than the value of 4.8 reported for beef heart ETP by Green and Wharton (13).

UQ Homologue in MB-ETP. It was of interest, in terms of comparative biochemistry, to determine the homologue of UQ functioning in the mitochondrial electron transfer chain of Phaseolus aureus. An experiment was performed on an extract eluted from the first thin layer chromatographic step described in the Methods section which consisted of a modified Craven's test (8). The blue color which developed with authentic UQ-10 exhibited a broad spectral peak in the region between 587 and 612 nm. The extract from MB-ETP exhibited a similar spectrum with a peak at approximately 610 nm, indicating the presence of an unsaturated quinone ring nucleus.

The homologue of UQ, or number of isoprenoid units at position 6, present in MB-ETP was investigated with the second thin layer chromatographic system described in the Methods section. This reversed phase partition system clearly separates the various naturally occurring UQ homologues from each other (fig 1). The MB-ETP extract migrates with an Rf identical to that of authentic UQ-10 (Rf = 0.13) while UQ-9 migrates more rapidly than UQ-10 in this system (Rf = 0.22). The Rf of UQ-4 was consistently lower than that of UQ-6 in the paraffin containing, reversed phase chromatographic system, presumably due to its slight water solubility in comparison to the higher homologues. We have not tested homologues lower than UQ-4.

The spectrum of the MB-ETP extract was obtained by cutting out the area from chromatograms such as that described in Methods from non-impregnated sheets developed with 3:1 benzene:chloroform, eluting with acetone, drying under a stream of nitrogen, dissolving in redistilled ethanol, and observing the spectral qualities with a recording spectrophotometer. The direct oxidized and reduced spectra are shown in figure 2. The characteristic peak for UQ at 275 nm in the oxidized and 290 nm in the reduced states (14) were observed.

Discussion

The purpose of this communication has been 2-fold, 1) to determine the presence and type of UQ present in the mitochondrial electron transfer chain of dark-grown Mung bean seedlings and 2) to determine its participation in electron transfer. Evidence in favor of the presence of UQ in such a preparation consists of the positive Craven's test on the partially purified lipid extract and its characteristic absorption spectra in the UV region. Although Craven's test is not specific for UQ homologues, Crane has reported (15) that of the terpenoid quinones found commonly in biological systems only the UQ homologues and β- and γ-tocopherolquinone give a positive reaction.

In addition, the type of UQ present in this plant species was established by its identical migration with authentic UQ-10 in a sensitive thin layer chromatographic system. The types of UQ homologues in plants are of interest for comparative reasons. In green plants the homologues UQ-8, UQ-9, and UQ-10 have been detected (15). Both plastoquinone and UQ have been reported to be present in Phaseolus radiatus seeds but the particular homologue of UQ present in this Phaseolus species has not been reported. No studies of this type have appeared on Phaseolus aureus. All of the UQ homologues 6 through 10 have been observed in species of fungi (15).

Except for 2 reports in abstract form (2,3) the function of UQ in mitochondrial electron transfer in higher plants has received little attention. The data presented above indicate that UQ may be a functional entity of plant mitochondrial systems in that UQ is reduced by both succinate and NADH, partially during steady-state conditions of electron transfer, and almost completely during anaerobiosis in the presence of substrate in MB-ETP. In addition, the amount of UQ present in MB-ETP is approximately equal to that found in beef heart submitochondrial particles in which UQ has been shown to be an obligatory component of the respiratory chain (16).

Many questions remain unanswered concerning UQ in plant mitochondrial systems since they have received so little attention with respect to lipid components. A major question of considerable importance concerns the detailed pathway of UQ reduction in such systems. Is UQ in plant mitochondria an obligatory juncture point for the oxidation

![Figure 2](https://example.com/figure2.png)

Fig. 2. Oxidized and reduced spectra of MB-ETP extract in ethanol. Reduction was obtained with a few grains of NaBH₄ and the spectrum was run again after 5 minutes.
of various flavoproteins as viewed by Klingenberg and Kroger (17) for intact animal mitochondria or does it participate in branched electron transfer pathways as viewed by Storey and Chance (18) for animal submitochondrial practices? It is hoped that the description for the preparation of submitochondrial particles in good yield from Phaseolus aureus described herein will stimulate research in this direction.

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Literature Cited