Electron Transport in Halophilic Bacteria: Involvement of a Menaquinone in the Reduced Nicotinamide Adenine Dinucleotide Oxidative Pathway

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The reduced nicotinamide adenine dinucleotide oxidative pathway of a halophilic bacterium was found to contain a light-sensitive (360 nm) compound, menaquinone-8, which serves as a cofactor in the nicotinamide adenine dinucleotide+-linked pathway.

Halophilic bacteria have been described as organisms requiring various concentrations of salt for growth and survival (10). Certain of these halophilic bacteria, referred to as "extreme halophiles" (2) fail to grow at salt concentrations below 15% (w/v). In addition, the apparent overall internal salt concentration, although different in ionic composition, is approximately equal to that of the external medium (6). Therefore, it is of interest to study electron transport in organisms possessing such a high intracellular salt concentration.

An electron transport scheme for halophilic bacteria has been proposed by several investigators (5, 9). These proposed schemes include cytochromes b, c, a, and α as well as flavoproteins but do not include a light-sensitive (360 nm) quinone as has been found by several investigators in nonhalophilic bacteria (3, 4, 7, 8). It is the purpose of this paper to present evidence indicating the presence of a light-sensitive (360 nm) menaquinone in the reduced nicotinamide adenine dinucleotide (NADH) oxidative pathway of an extreme halophile.

The organism, an orange-pigmented extreme halophile originally isolated from the Great Salt Lake, Utah (R. Nachum, Ph.D. thesis, Univ. of Southern California, 1969), was grown at 45°C in a standard complex medium as previously described (R. Nachum, Ph.D. thesis, Univ. of Southern California, 1969) and harvested by centrifugation. A salt solution (SS; 0.04 M MgCl₂ in 2.5 M NaCl) was used to wash and suspend the cells. The organisms were disrupted by sonic oscillation, and a cell free extract obtained by centrifugation at 14,000 × g for 30 min was then separated into supernatant and particulate fractions by centrifugation at 144,000 × g for 60 min. The resulting electron transport particles were washed and resuspended in SS.

Irradiated particles were obtained by exposing the particulate suspension to 360 nm light from 12 GE lamps, type BLB ("black light"), in a rotary radiation apparatus for 25 min (8). Protein determinations were carried out using the turbidometric method of Stadman et al. (13).

A sample of particles was divided into two samples, each containing 66.8 mg of protein. One sample was irradiated, and the lipids were extracted from both fractions as previously described (12). The resulting materials were dissolved in petroleum ether and chromatographed on a column of Permutit, Folin (Fisher Scientific Co.). The columns were eluted stepwise with 100 ml samples of 4, 10, 25, and 100% (v/v) diethyl ether in petroleum ether (12) and the samples were taken to dryness. The resulting lipid fractions were further purified using adsorption chromatography on silica gel G plates impregnated with Rhodamine 6G with 12% butyl ether in hexane as a solvent (12). A comparison of corresponding bands from irradiated and nonirradiated particles indicated that the lipids were similar, with one notable exception. A band which migrated with a R₅ of approximately 0.4 and which appeared to contain a relatively much greater amount of material in the nonirradiated as compared to the
irradiated samples was found in fractions eluted with 4 and 10% diethyl ether in petroleum ether. The corresponding bands were eluted with ether, taken to dryness, and the ultraviolet spectrum was measured in isooctane using a Cary (model 11) spectrophotometer. The spectra of the two corresponding bands are shown in Fig. 1. Curve 1 represents the spectrum of the compound from the unirradiated particles and has absorption maxima typical of a 2,3-disubstituted 1,4-naphthoquinone (11). The quinone concentration was calculated to be approximately 2.24 nmoles/mg of protein (E\textsubscript{1} %\textsubscript{cm} = 411 at 268 nm). After irradiation at 360 nm, the compound no longer displayed peaks at 242, 248, 260, and 268 nm indicating at least partial structural decomposition of the compound (Curve 2). The amount of quinone present in the irradiated sample could not be determined spectrophotometrically since absorption peaks necessary for extinction coefficient calculations were no longer present; however, the amount of particles used for extraction and chromatography was the same in both the irradiated and unirradiated samples.

Endogenous quinone used in the restoration experiments below was extracted from whole cells, as outlined above, except that additional purification was carried out using reversed-phase chromatography on cellulose plates and silica-gel G plates impregnated with silver nitrate as previously described (12).

The reaction systems used in measuring oxidative activity of irradiated and nonirradiated electron particles and in restoration experiments consisted of the following: particles, 7.5 mg of protein; 200 μmoles of HEPES-KOH (N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid) buffer, pH 7.6; 0.05 ml of Asolectin (2.0% w/v) with or without quinone as indicated; and where indicated, 100 μmoles of NADH; 10 μmoles of KCN; 1.3 μmoles of vitamin K\textsubscript{1}; 1.6 μmole of halophile (endogenous) quinone; supernatant, 2.6 mg of protein; and SS to a final volume of 3.0 ml. The quinones were suspended in Asolectin by the procedure previously described (1). Oxygen uptake was measured with a Clark oxygen electrode.

The oxidation of NADH by the particulate fraction from the halophile was sensitive to cyanide (Table 1). This observation is in accord with the proposed schemes of Lanyi (9) and Cheah (5) in that NADH oxidation proceeds through a KCN-sensitive terminal oxidase. It is of interest that the soluble fraction obtained after removal of the particles failed to oxidize NADH and did not affect the oxidation of this substrate by the particulate fraction. After irradiation, the particles exhibited a diminished rate of NADH oxidation (Table 1), approximately 69% below that of the rate with unirradiated particles. This residual activity was not cyanide-sensitive.

Restoration of oxidative activity with particles subjected to irradiation occurred with vitamin K\textsubscript{1} and the endogenous quinone (Table 2). Vitamin K\textsubscript{1} was used since it is a 2,3-disubstituted 1,4-naphthoquinone, it can be obtained commercially, and it has been shown to restore oxidation to irradiated (360 nm) electron transport particles (3, 4, 7, 8). Vitamin K\textsubscript{1} partially restored

![Fig. 1. Spectra of light-sensitive quinone isolated from irradiated and nonirradiated electron transport particles. (1) Nonirradiated; absorption maxima at 242, 248, 260, 268, 326, and a shoulder at 238 nm. (2) Irradiated.](http://jb.asm.org/)
the oxidative activity to a level approximately 53% of the original value, whereas the endogenous quinone restored the activity to a level approximately 60% of the original. The restoration of oxidative activity occurred by a cyanide-sensitive pathway with either vitamin K₃ or the endogenous quinone (Table 2). The inhibition by cyanide of the restored oxidative activity using K₃ or the endogenous quinone was approximately the same, 87%.

Since the endogenous quinone appears to function in the NADH oxidative pathway, studies were undertaken to determine the chemical structure of this quinone. The ultraviolet spectrum was characteristic of a 2,3-disubstituted 1,4-naphthoquinone (Fig. 1, Curve 1; 11). Since these quinones can vary in the length of the C₃ isoprenoid side chain and degree of saturation, two chromatography systems were used to separate quinones differing in the length and degree of saturation. Silica-gel G plates impregnated with silver nitrate and reversed-phase chromatography on Kieselguhr were used as previously described (12) with authentic quinone markers. The endogenous quinone was found to comigrate with the authentic MK₄ marker (Fig. 2A, 2B). Thus it would appear that the saturation and length of the isoprenoid side chain is similar to that of MK₄. This observation is in accord with the data of Tornabene et al. (14) who found that the quinone present in the extreme halophile Halobacterium curtirubrum was MK₄. The infrared and mass spectra (not shown) of the endogenous quinone were also in accord with the data of Tornabene et al. indicating the natural quinone from this halophile is MK₄.

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Table 2. Restoration of oxidative activity using vitamin K₃ and the endogenous naphthoquinone isolated from the organism

<table>
<thead>
<tr>
<th>Reaction system</th>
<th>Additions</th>
<th>O₂</th>
</tr>
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<tbody>
<tr>
<td>Irradiated particles</td>
<td>NADH + K₃</td>
<td>1.32</td>
</tr>
<tr>
<td>Irradiated particles</td>
<td>NADH + K₃ + KCN</td>
<td>0.69</td>
</tr>
<tr>
<td>Irradiated particles</td>
<td>NADH + NQ⁺</td>
<td>1.41</td>
</tr>
<tr>
<td>Irradiated particles</td>
<td>NADH + NQ + KCN</td>
<td>0.71</td>
</tr>
</tbody>
</table>

a Conditions and concentrations are given in the text. The preparation used was the same as that given in Table 1.

b Expressed as micromoles per minute per micromole of quinone.

c NQ, Endogenous naphthoquinone, MK₄.

Fig. 2. Chromatographic behavior of the endogenous quinone (Q). Silver nitrate chromatography (A) and reversed-phase chromatography (B) were used with authentic quinone markers.

LITERATURE CITED