RESTORATION OF ELECTRON TRANSPORT IN ULTRAVIOLET-IRRADIATED MEMBRANES OF *AEROBACTER AEROGENES*

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Received 2 March 1971

1. Introduction

The ubiquinone-8 mediated electron transport to nitrate and to oxygen in membranes from *Aerobacter aerogenes* is effectively inhibited by irradiation with ultraviolet light [1]. In previous experiments attempts to restore the electron transport in irradiated membranes by addition of the natural ubiquinone or related homologues, have been unsuccessful [1]. This failure could be due to the following causes: a) ultraviolet irradiation not only destroys or alters the endogenous quinone [see 2], but also produces other effects on the electron transport system, e.g. by altering protein structures or by breaking metal-protein bonds [3]; b) sticking of the modified ubiquinone molecules in the irradiated membranes prevents the proper incorporation and functioning of newly added quinone.

In the present study, extraction of the modified ubiquinone from irradiated membranes with pentane and subsequent reincorporation of intact ubiquinone was found to result in a restoration of the NADH oxidation to a large extent. The results obtained show that the effect of ultraviolet irradiation on electron transport in *A. aerogenes* is mainly attributable to destruction of endogenous ubiquinone, but also that in some cases alteration of protein structures can occur.

2. Methods

A. aerogenes strain S 45 was cultured anaerobically in a minimal nitrate plus NH_4^+ medium [1] to a cell density corresponding to a dry weight of 120 mg per liter. A cell-free extract was prepared by sonic disintegration of the bacteria, and subsequently the membrane fraction was obtained by centrifugation at 120,000 *g* for 1 hr [4]. Washed membranes were resuspended (4–5 mg protein/ml) in 0.065 M phosphate buffer, pH 7.0, and irradiated at 4° with light of 354 nm for 90 min in the same manner as described previously [1].

Ubiquinone was extracted from irradiated and untreated membranes in essentially the same manner as described for mitochondria by Szarkowska [5]. Lyophilized membranes (50–75 mg protein) were suspended in 70 ml of *n*-pentane by gentle homogenization for 5 min, and the suspension was swirled at 4° for 15 min [1]. This procedure was repeated until ultraviolet absorbing material no longer appeared in the extract.

Ubiquinone incorporation was carried out by the method of Ernster et al. [6], described for submitochondrial particles. Dried, quinone-depleted membranes (25–30 mg dry weight) were resuspended in 2 ml of *n*-pentane containing ubiquinone-8 at a final concentration of 1.15 mM, followed by homogenization. After removal of excess ubiquinone-8 by centrifugation, the membranes were dried and resuspended in 0.065 M phosphate buffer pH 7.0 [1].

NADH oxidase and NADH-linked nitrate reductase activities were measured as described previously [1]. Nitrate reductase activity with reduced benzyl viologen as electron donor was assayed as described elsewhere [4]. Oxygen uptake was measured polarographically with a Clark oxygen electrode at 30° and proteins were determined by the Lowry technique.

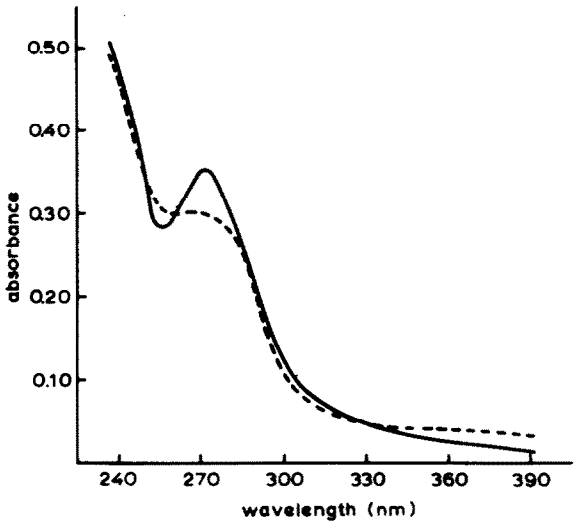

Fig. 1. Absorbance spectra of ubiquinone-8 extracted with pentane from untreated (——) and irradiated (- - -) membranes. Ubiquinone was extracted as described in the Methods section. The extracts were chromatographed on a column of Brockmann grade III acid-washed alumina (M. Woelm, Eschwege, Germany; anionotropic) developed by stepwise elution with successively 0, 2, 4, and 6% (v/v) solutions (100 ml) of diethylether in light petroleum (b.p. 40–60°). Ubiquinone, found to be present in the 4% ether fraction, was further purified by chromatography on silica gel G plates impregnated with Rhodamine 6G with benzene as developing solvent, followed by reversed-phase chromatography on silica gel G plates impregnated with a 10% (v/v) paraffin solution in light petroleum (b.p. 60–80°). Ubiquinones with known isoprenoid chain length were used as markers. After development of the plates with acetone–water (95:5, v/v) one band, comigrating with the ubiquinone-8 marker, was found in the extracts of both irradiated and untreated membranes. The bands were quickly taken up with ether. After removal of the ether, the quinone was dissolved in pentane and its absorbance spectrum was measured in a Unicam SP.800 spectrophotometer.

3. Results and discussion

A membrane suspension (4.7 mg protein/ml) was divided into two batches, one of which was irradiated with ultraviolet light. After lyophilization of both batches, endogenous ubiquinone-8 was extracted with pentane, purified by chromatography, and the absorbance spectra were measured (fig. 1). Irradiation appears to cause a structural modification of the endogenous ubiquinone, as judged from the alteration in spectral properties. Similar changes in spectral properties are observed after irradiation of an ethanolic solution of ubiquinone [2].

Table 1
Effect of ultraviolet irradiation on oxidase systems in membrane from *A. aerogenes* grown under anaerobic conditions.

Substrate	Final concn. (mM)	O_2 uptake with	
		Untreated membranes	Irradiated membranes
NADH	0.8	815	286
Succinate	0.8	74	29
Ascorbate plus DCIP	7.0 0.3	5.9	55

Membranes were prepared and irradiated as described in the Methods section. The activities are expressed as natoms of oxygen consumed per min per mg of protein. The NADH and succinate oxidase activities were measured polarographically at 30° in a 3.0 ml medium, containing 185 μmoles sodium-potassium phosphate buffer (pH 7.0), about 1 mg of protein and the indicated substrates. The ascorbate-DCIP oxidase activity was determined under the same conditions in a 1.3 ml medium, containing 33 μmoles sodium-potassium phosphate buffer (pH 7.4), 6 μmoles $MgCl_2$, 1 μmole EDTA and 0.15% Triton X-100 (according to a suggestion of Dr. L.J.M. Eilermann, Laboratory of Biochemistry, B.C.P. Jansen Institute, University of Amsterdam).

Besides the spectral alteration in the quinone, irradiation causes an inhibition of oxidase activities and of the NADH-linked nitrate reductase activity in membrane preparations, as shown in tables 1 and 2. On the other hand, the ascorbate-DCIP system, in which electrons are directly transferred to the cytochrome region of the electron transport chain, was not influenced by ultraviolet light.

In order to restore the electron transport from NADH to oxygen and to nitrate, the irradiated membranes were lyophilized and the modified ubiquinone was extracted with pentane, followed by reincorporation of intact ubiquinone-8 into the depleted membranes. Lyophilization of the membrane preparations caused a loss of NADH oxidase and, in particular, of NADH-linked nitrate reductase activity. This loss could be minimized by lyophilizing the membranes for as short a period as necessary to obtain complete dryness. Table 2 demonstrates that reincorporation of ubiquinone-8 into irradiated and subsequently pentane-extracted membranes resulted in a strong restoration of the electron transport from NADH to oxygen, equivalent to about 90% of the activity ex-

Table 2
Restoration of NADH-linked electron transport in irradiated membranes by ubiquinone-8 reincorporation.

Membrane preparations	NADH oxidase activity		NADH-linked nitrate reductase activity	
	Non-irradiated	Irradiated	Non-irradiated	Irradiated
Normal	1165	365	464	140
Lyophilized	1050	335	370	114
Lyophilized-extracted	255	46	65	9
Lyophilized-extracted plus ubiquinone	588	524	212	55

The preparation and lyophilization of the membranes, and the extraction and reincorporation of ubiquinone-8 were performed as described in the Methods section. The enzymatic activites, expressed as nmoles of NADH oxidised per min per mg of protein, were measured by recording the decrease in absorbance at 340 nm in a Unicam SP.800 spectrophotometer at 30°. The NADH-linked nitrate reductase activity was determined under argon atmosphere in Thunberg cuvettes in the presence of 3.0 mM KNO_3. In all determinations the reaction mixture contained 0.2 mM NADH as substrate, 65 mM sodium-potassium phosphate buffer (pH 7.0) and 0.3–0.4 mg protein.

hibited by non-irradiated membranes after pentane extraction and reincorporation of ubiquinone-8. These results indicated that destruction or modification of endogenous ubiquinone is the main cause for the decrease in oxidase activites produced by ultraviolet irradiation. The insensitivity to ultraviolet light of the ascorbate-DCIP system, which does not need ubiquinone, supports this conclusion (table 1).

On the other hand, electron transport from NADH to nitrate in irradiated membranes could hardly be restored by reincorporation of ubiquinone-8. The restoration of this activity in irradiated membranes corresponds to only 25% of the restored activity in non-irradiated membranes. This lack of restoration is apparently due to the inactivating effect of ultraviolet light on the terminal nitrate reductase, since it was found that nitrate reductase activity measured with reduced benzyl viologen (which transfers electrons directly to the enzyme) as electron donor, was also strongly inhibited by irradiation of membranes. Therefore, besides the destruction of ubiquinone as a main effect, ultraviolet irradiation may also lead to inactivation of certain enzymes.

Acknowledgements

This work was supported in part by the Netherlands Foundation for Chemical Research (S.O.N.) and the Netherlands Organization for the Advancement of Pure Research (Z.W.O.). We thank Dr. J. van 't Riet for valuable discussions and Mrs. Eelkje van Brussel for her collaboration in some of the experiments. Ubiquinone-8 was a gift from F. Hoffman-La Roche and Co., Basle, Switzerland.

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