PRELIMINARY NOTE

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Redox reactions of ubiquinone in *Rhodospirillum rubrum*

In an attempt to elucidate the mode of action of ubiquinone in the electron transport system of photosynthetic bacteria, studies have been made of the redox reactions of endogenous and exogenous ubiquinone in whole cells and chromatophores of *Rhodospirillum rubrum*.

R. rubrum was grown anaerobically in the light in the medium described by GELLER[1]. The harvested cells were suspended in 0.1 M Tris–HCl buffer, pH 7.8. Chromatophores were prepared by disintegration of the cells in a French pressure cell followed by centrifugation at 20000 × *g* for 30 min. The residue was discarded and the supernatant centrifuged at 60000 × *g* for 1 h. The sedimented chromatophore fraction was suspended in 0.1 M Tris–HCl, pH 7.8, and in some experiments, was used as such. Washed preparations were prepared by recentrifugation at 60000 × *g* for 1 h followed by resuspension in the Tris buffer. Dry weight and chlorophyll content were determined on the whole cell and chromatophore suspensions as described by COHEN-BAZIRE *et al.*[2].

To measure the steady-state redox level of the endogenous ubiquinone, the whole cell or chromatophore preparation (0.5 ml containing 0.2–0.4 mg chlorophyll) was shaken mechanically in a test-tube (1.5 cm diameter) in a glass sided water bath maintained at 25°. The tube was illuminated by two 150-W spot lamps on opposite sides of the tank to give a total light intensity of approx. 4000 ft-candles at the tube. Anaerobic conditions were maintained when necessary by blowing a stream of oxygen-free nitrogen into the tube. After shaking the tube for 3 min under a particular condition (light or dark, aerobic or anaerobic), the reaction was terminated by the rapid addition of 5 ml of a mixture of methanol and acetone (50:50, v/v) at room temperature. This addition was followed immediately by 5 ml of 40–60° light petroleum. The tube was shaken for 1 min and after separation of the layers, the light petroleum layer was removed. The extraction was repeated twice more with 4-ml portions of light petroleum. The combined light petroleum extracts were then partitioned with 5 ml of 95 % methanol. After removal of the solvent *in vacuo*, the residue was dissolved in 3 ml ethanol and the spectrum determined in the 225–350 mμ range before and after the addition of sodium borohydride. The ubiquinone content and redox state were then calculated as previously described[3].

Experiments on the photoreduction of exogenous ubiquinone (and other quinones) coupled to the photooxidation of reduced cytochrome *c* were carried out by a modification of the method described by ZAUGG[4]. Oxidized quinone and reduced cytochrome *c* were used in the initial reaction mixture and the reaction was followed in the Aminco–Chance dual-wavelength spectrophotometer at the wavelength pair 550–545 mμ. Actinic light was provided by a tungsten lamp in a side-illumination attachment equipped with a filter (Wratten 29) transmitting wavelengths above

TABLE I

REDOX LEVELS OF ENDOGENOUS UBIQUINONE IN WHOLE CELLS AND CHROMATOPHORES OF *Rhodospirillum rubrum*

Experimental details described in text. PMA stands for phenylmercuric acetate.

Preparation	*Light or dark*	*Aerobic or anaerobic*	*Reduction of total ubiquinone* (%)		
			No addition	*+ PMA*	*+ KCN*
Whole cells	Dark	Aerobic	26	29	64
	Light	Aerobic	27	29	58
	Dark	Anaerobic	56	67	71
	Light	Anaerobic	53	64	70
Chromatophores	Dark	Aerobic	39	—	—
	Light	Aerobic	20	—	—
	Dark	Anaerobic	57	—	—
	Light	Anaerobic	23	—	—

600 mμ. To prevent the actinic light affecting the photomultiplier a guard filter (Wratten 58) was used.

The results of the experiments on the redox states of endogenous ubiquinone in whole cells and chromatophores are summarized in Table I. It will be seen that under aerobic conditions in the light or dark the quinone is predominantly oxidized whereas when the system is made anaerobic it becomes extensively reduced. Addition of phenylmercuric acetate to whole cells increases slightly the reduction in the anaerobic state while in the presence of KCN there is an increased reduction in the aerobic state. It will be noted that in each case, the effect of light in the anaerobic

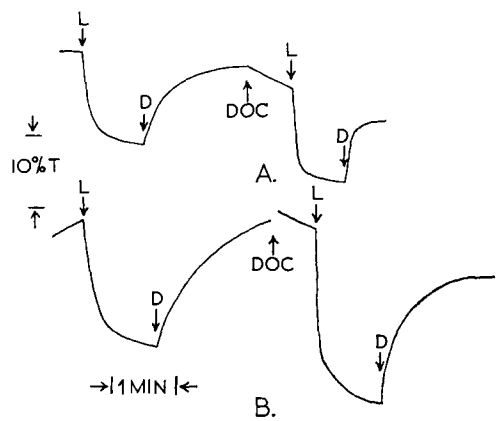

Fig. 1. Photooxidation of cytochrome *c* coupled to reduction of ubiquinone and other quinones in *R. rubrum* chromatophores. L = light, D = dark, DOC = deoxycholate. A, reaction in presence of Q-2; B, reaction in presence of 2-methoxy-6-propyl-1,4-benzoquinone. Reaction mixture consisted of: 250 μmoles Tris–HCl buffer, pH 7.8; 72 μmoles reduced cytochrome *c*; 400 mμmoles quinone in 0.02 ml ethanol; washed chromatophore preparation containing 11 μg bacteriochlorophyll; 2 mg sodium deoxycholate added where indicated. Total reaction vol., 2.75 ml; temp., 23°. Rate of change of percentage transmission measured at wavelength pair 550–545 mμ. Oxidation is indicated by a downward deflexion. Further experimental details are described in the text.

state is to decrease the extent of reduction. In the case of the chromatophores (an unwashed preparation containing endogenous hydrogen donor) the effect of the light in increasing the oxidation level of endogenous ubiquinone is clearly seen.

The results of experiments on the coupled reaction between added quinone and cytochrome *c* are depicted in Fig. 1. A rapid photooxidation of reduced cytochrome *c* occurred in both the presence of the ubiquinone homologue, Q-2, and of 2-methoxy-6-propyl-1,4-benzoquinone as acceptors. Addition of deoxycholate increased the rates of the light and dark reactions with both quinones. Thus it appears that this system is not specific for ubiquinone homologues but that other benzoquinones may also participate; other quinones tested which proved to be active included tetramethyl-1,4-benzoquinone and 2,6-dimethyl-1,4-benzoquinone. However, another system of the chromatophore fraction exhibited a much greater specificity towards ubiquinone homologues. This is the succinate oxidase system which was markedly stimulated by the addition of ubiquinone homologues. The mechanism of this effect has not yet been elucidated but it is likely to be either an activation effect similar to that in mitochondrial preparations[5] or a short circuiting of part of the electron transfer chain. However, the quinones, other than the ubiquinone homologues, which were active in the coupled system did not exert a stimulatory effect on the succinate oxidase system.

The work of CLAYTON[6] and ZAUGG[4] suggests that ubiquinone in photosynthetic bacteria acts close to the chlorophyll system and that the primary photochemical act involves the transfer of electrons from chlorophyll to ubiquinone. However, the fact that the endogenous quinone is reduced by hydrogen donors in the dark in chromatophores and whole cells indicates that it must also be placed close to the flavoprotein dehydrogenases as in the mitochondrial system. The present work has confirmed the finding that added ubiquinone is photoreduced by chromatophores but it has also suggested that since other quinones function in the coupled system, the added quinone may be acting as a relatively non-specific acceptor at a site different from that of the endogenous ubiquinone. Furthermore, the results have shown that the effect of light on the whole cell or chromatophore is to induce an oxidation, rather than a reduction of the endogenous ubiquinone, which has already been extensively reduced in the dark by an endogenous hydrogen donor.

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