EFFECTS OF EXTRACTION AND REPLACEMENT OF UBIQUINONE UPON THE PHOTOCHEMICAL ACTIVITY OF REACTION CENTERS AND CHROMATOPHORES FROM *RHODOPSEUDOMONAS SPHERIODES*

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Received 17 May 1974

1. Introduction

Ubiquinone (UQ) has been implicated as a primary photochemical electron acceptor in bacterial photosynthesis. Evidence for this has included light-induced optical absorbance changes suggesting disappearance of the oxidized form of UQ [1] and formation of the anionic semiquinone, UQ^- [2,3], and an electron spin resonance signal appropriate for the semiquinone [4,5]. UQ also functions as a secondary electron acceptor coupled to the primary photochemical system [2,6,7].

Dried preparations from photosynthetic bacteria have been subjected to extraction with hydrocarbon solvents such as iso-octane in order to study the effects of depleting UQ [2,6–10]. Such extraction removes the component of UQ that functions as a secondary electron acceptor, and this function can be restored by adding back UQ [2,9]. In these experiments the capacity for primary photochemistry, as manifested by the oxidation of bacteriochlorophyll (BChl), was not attenuated, casting some doubt on the role of UQ in the photochemistry.

We show here, using chromatophores and reaction centers (RC's) prepared from *Rhodopseudomonas spheroides,* that if a trace of methanol is added to the iso-octane used for extraction, the primary photochemistry can be abolished and can be restored by adding back purified quinones. These results show that UQ is important for photochemical activity. They have no bearing on the possible role of iron (as 'photoredoxin') [11] as part of the primary electron accepting system in bacterial photosynthesis.

2. Materials and methods

Purified chromatophores and RC's were prepared from *Rps. spheroides* strain R-26 as described by Clayton [12] and Clayton and Wang [13] respectively. The preparations were dialyzed against 0.01 M Tris–Cl pH 7.6 (hereafter called buffer) prior to drying them as films on glass surfaces. RC's were dried under argon on the inner surfaces of optical absorption cuvettes; chromatophores were dried in air on microscope slides.

Films of RC's were extracted with successive portions of iso-octane (2,2,4-trimethylpentane) containing 0.1% methanol, by filling the cuvette and allowing each portion to stand 10 min. The extracts were assayed for UQ by evaporating them, redissolving the residues in methanol, and measuring absorption spectra of the methanolic solutions before and after adding sodium borohydride. We computed amounts of UQ from $\Delta\epsilon = 12.2\ mM^{-1} \cdot cm^{-1}$ at 275 nm [14]. Following extraction, the dried RC's could be redispersed in buffer containing 0.3% lauryl dimethyl amine oxide (LDAO). UQ could then be added as a concentrated ethanolic solution, keeping the final concentration of ethanol less than 1%.

Extraction of the films caused some of the RC's to become denatured; extraction sufficient to abolish 90% of the photochemical activity caused about 50% denaturation. In the redispersed aqueous sample the denatured protein was insoluble and could be removed by centrifugation, leaving a clear suspension of undenatured RC's. Some BChl arising from denatured RC's remained in solution; it could be bleached irre-

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versibly by strong illumination before any quantitative measurement (such as those in fig. 1) were made. Final aqueous dispersions of extracted and unextracted films were adjusted to have the same optical density (OD) at 860 nm for comparative purposes.

Films of purified chromatophores were immersed in iso-octane to deplete the secondary electron acceptor pool, then in iso-octane containing 0.3% methanol to attenuate the primary photochemistry, and finally in isooctane containing 1 mM quinone to restore both the primary and secondary functions. The entire cycle could then be repeated with the same film. Restoration was made with UQ-50 (Coenzyme Q_{10}), UQ-30 (Coenzyme Q_6), and 1,4-naphthoquinone.

Denaturation of the chromatophores in these films appeared to be negligible; neither drying nor extraction had significant effects on their absorption spectra.

Optical absorption spectra were measured with a Cary 14R Spectrophotometer, using the 'IR-1' mode for measurements in weak light and the 'IR-2' mode to provide strong white illumination of the sample during measurement. Kinetic measurements of light-induced absorbancy changes were made on RC's in our laboratory using a home-made split-beam spectrometer [2], and on chromatophores in Dr W. L. Butler's laboratory using a dual-wavelength spectrometer based on Aminco-Chance optical and mechanical components. Relative values of quantum efficiency were estimated (within ± 20%) as the initial 'light on' slopes of ΔOD versus time. These measurements were made at 862 nm for RC's, and at either 425–403 nm or 603–590 nm (dual wavelength) for chromatophores; in every case the change signified photochemical oxidation of BChl [15]. Exciting light was at 800 nm. For estimation of quantum efficiency the intensity of the exciting light and the scales of OD and time were adjusted to give an initial slope near 45°C.

3. Results and discussion

Fig. 1 shows absorption spectra of RC's (0.9 μM, see [16] in aqueous suspension, following exhaustive extraction with iso-octane + 0.1% methanol. Comparing curves I and II (Ir-1 and IR-2 modes respectively) we see that the extracted sample was unresponsive to strong light except for a slight irreversible bleaching. Photochemical activity was restored fully when 10 μM

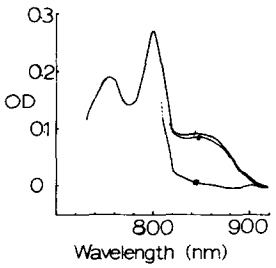

Fig. 1. Reaction centers from *Rps. spheroides* R-26 were dried, extracted with iso-octane containing 0.1% methanol, and redispersed in 0.01 M Tris–Cl, pH 7.6, with 0.3% lauryl dimethylamine oxide. Absorption spectra were measured in weak light (curve I) and while exposing the sample to strong light (curve II). Ubiquinone, 10 μM, was then added and the measurement in strong light repeated (curve III). An unextracted control sample gave results like those of curves I and III. Weak and strong light were provided respectively by the IR-1 and IR-2 modes of a Cary 14R Spectrophotometer.

UQ-30 had been added; the spectrum measured in the IR-2 mode (curve III) then showed complete bleaching of the 865 nm band. Control samples that had not been subjected to extraction gave spectra like those of curves I and III. The kinetics of the reaction in reconstituted RC's were like those in the unextracted control.

In another series of measurements with a sample containing 1.2 μM inactive (extracted) RC's, the addition of 0.6 μM UQ-30 restored half of these RC's to photochemical activity and 2.6 μM UQ-30 gave more than 90% restoration.

Table 1 shows how photochemical activity was lost in RC's during progressive extraction of UQ. This sample consisted of 7.5 nmoles of RC's which yielded a total of 7.6 nmoles of UQ in five consecutive extractions. Note that the loss of photochemical activity paralleled approximately the extraction of UQ, when an irreversible component of the bleaching at 865 nm was discounted. The irreversible bleaching may have been due to pigment from denatured RC's remaining in the film.

Fig. 2 shows light-induced absorbance changes in a film of purified chromatophores, measured at 425–403 nm. Similar results were obtained at 603–590 nm. The successive traces show how iso-octane alone removed secondary electron acceptors (faster back-reaction in traces *b* and *e*), how iso-octane + 0.3% methanol attenuated the photochemistry (*c* and *f*), and how photochemical activity *and* a semblance of

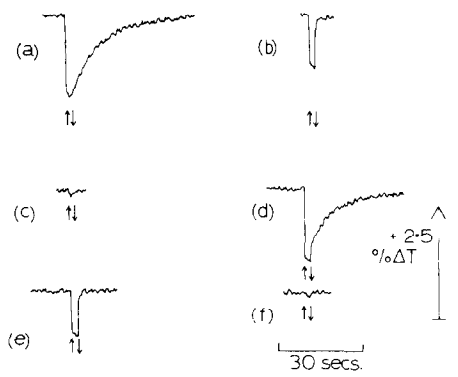

Fig. 2. Purified chromatophores from *Rps. spheroides* R-26 were dried onto a microscope slide and light-induced absorbance changes of the dried film were examined with a dual wavelength spectrometer at 425–403 nm. Exciting light, 2 sec at 800 nm, about 1 mw/cm², was applied as shown by the arrows. The film was subjected to a sequence of extraction and infusion; traces *(a)* – *(f)* show the behavior after successive steps: *(a)* No treatment. *(b)* 24 hr in iso-octane. *(c)* 15 hr in iso-octane + 0.3% methanol. *(d)* 75 min in iso-octane + 1 mM ubiquinone-30. *(e)* 20 min in iso-octane. *(f)* 11 hr in iso-octane + 0.3% methanol.

the 'natural' reaction kinetics could be reconstituted by adding back UQ-30 to the extracted film (trace *d*). UQ-50 gave the same result as UQ-30. Naphthoquinone restored photochemical activity, but with a faster back-reaction.

In both RC's and chromatophores, the quantum efficiency of the reaction in reconstituted samples was equal to that in the corresponding unextracted sample, within ± 20%.

We have shown that following extraction of RC's or chromatophores, a photochemical reaction similar (perhaps identical) to the native one can be restored by adding back UQ. We do not know why the component of UQ associated with primary photochemistry is extracted less easily than the 'secondary' component. Bolton and Cost [17] have suggested that iron and UQ form a primary electron-accepting complex in which the UQ is bound more firmly. They gave evidence that in RC's depleted of iron, the 'primary' UQ can be extracted with pure iso-octane. Hopefully the functional relationships between BChl, iron and UQ can be explored more fully now that we are able to extract the 'secondary' and 'primary' UQ selectively.

Acknowledgements

Steven Drews provided expert technical assistance. We thank Dr W. L. Butler of the University of California, San Diego for the hospitality of his laboratory during the stay of one of us (RKC) on a John Simon Guggenheim Memorial Fellowship. This work was supported by Contract No. AT(11-1)-3162 with the U.S. Atomic Energy Commission and Grant No. GB36591X from the National Science Foundation.

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Table 1
Loss of photochemical activity in a dried film of reaction centers (7.5 nmoles) extracted to remove ubiquinone

Extraction number	Percent of maximal ΔOD at 865 nm		Cumulative nmoles of UQ extracted
	Total	Irreversible	
0	100	<5	0
1	64		2.2
2	50		4.2
3	32		6.1
4	28		6.9
5	26	20	7.6

Activity was measured by the light-induced bleaching at 865 nm

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