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Quinones of Athiorhodaceae

Current uncertainties in assignment of function to quinones in bacterial photosynthesis[1] very probably reflect fluctuations in chromatophore quinone content. It is common laboratory experience that in the photoheterotrophic Athiorhodaceae, cell composition can vary greatly in most species if culture conditions are not controlled rigorously. Quinones, which are present in large quantities—much in excess of other electron transport components in chromatophores—may be particularly susceptible to variation in kind and quantity. As an example, we may cite the experiments of SUGIMURA AND RUDNEY[2] who noted marked decreases in ubiquinone content of *Rhodospirillum rubrum* when grown either photosynthetically in the presence of diphenylamine, or aerobically in the dark.

There appears to be no systematic data available on the effects of various growth parameters in relation to quinone nature and distribution in chromatophores. Accordingly, we have initiated studies designed to elaborate these effects. Ultimately we hope such researches will supply a basis for nutritional manipulation of organisms to produce standard source material for investigations of primary processes in bacterial photosynthesis. We present herewith preliminary findings on quinone distribution in eleven species of Athiorhodaceae and one (metabolically related) species, *Rhodomicrobium vanniellii*, grown on a single, well-defined medium[3] under controlled environmental conditions, so that comparisons are valid.

In experiments on stationary phase organisms, cultures were grown in a 2-l Pyrex bottle, diameter 12.5 cm, immersed in a glass thermostat bath illuminated by two 75-W Philips floodlights placed 25 cm from the center of the bottle. Except for *Rhodopseudomonas capsulatus*, which was grown at 28° because of adverse effects for this organism at 30°, the temperature for all cultures was maintained at 30°. A N_2–CO_2 (95:5, by vol.) sterile gas mixture was bubbled through the culture and, together with the use of a magnetic stirrer, provided adequate agitation while minimizing the usual rise in pH owing to release of alkali during assimilation of the organic substrates. The initial pH (6.8) never rose more than one pH unit by the time of harvest (24 h after attainment of stationary phase). To monitor cell densities, we measured absorbance with a Cary spectrophotometer, Model 14, at 1200 mμ. At this wavelength, interference by photopigments was minimal and turbidity measured accurately reflected cell mass—an important consideration because photopigment composition was found to vary entering the stationary phase (H. DE KLERK AND M. D. KAMEN, unpublished). To correct for any non-linearity at 1200 mμ, measurements were made routinely by dilution to a standard absorbance of 0.1 and the appropriate dilution factor applied to record cell mass. Species used are listed in Table I.

Nitrogen was determined by a modified Nessler procedure, according to LANG[4]. (Results referred to protein nitrogen content will be presented elsewhere.) Bacteriochlorophyll was extracted and measured by its absorbance in methanol[5] using the absorption maximum at $\lambda = 772$ mμ, for which the extinction coefficient was taken as 60 $mM^{-1} \cdot cm^{-1}$. In the one case of *Rhodospirillum viridis*, no estimate was made because its bacteriochlorophyll appeared atypical with λ_{max} at 794 mμ. Total quinone analyses were made on lyophilized bacteria, using a new method developed by POWLS

TABLE I

QUINONES IN ATHIORHODACEAE

The various species shown are designated as follows: *Rps. gelatinosa* (Van Niel, No. 2.2.22), *Rhodospirillum molischianum* (Stanier, Berkeley 1), *R. viridis* (Ehmjellen N), *Rhodospirillum fulvum* (Pfennig KK), *R. rubrum* (ATCC, S-1), *R. rubrum* (G-9) (Newton), *Rps. spheroides* (Van Niel, No. 2.4.2), *Rps. capsulatus* (Van Niel, No. 2.3.2), *Rm. vannielli* (Van Niel). Rhodopseudomonas sp. No. 2761 designated as *R. fulvum* by Professor N. PFENNIG (private communication) appears to be a species other than *fulvum*, based on subsequent studies on intracellular structure by Dr. G. COHEN-BAZIRE (unpublished) and composition of electron transport system (H. DE KLERK, unpublished). Rhodopseudomonas sp. ("FRNY") is a recent isolate, as yet unclassified, but in the genus Rhodopseudomonas (H. DE KLERK, unpublished).

Species	*Quinone*		*Menaquinone*		*BChl content**	*BChl:Q* ($\mu M : \mu M$)
	Type	*Content**	*Type*	*Content**		
Rps. gelatinosa	Q_8	1.9	MK_8	0.66	7.7	4.3
Rhodospirillum sp. No. 2761	Q_8	2.7	MK_8	0.92	15.6	5.7
R. molischianum	Q_9	2.7	MK_9	0.72	25.5	9.4
R. viridis	Q_9	3.0	MK_9	1.19	**	**
R. fulvum	Q_9	3.8	MK_9	0.91	29.8	7.9
R. rubrum	Q_{10}	6.3	$Rhodoquinone_{10}$		13.0	2.0
R. rubrum (G-9)	Q_{10}	5.3	Rhodoquinone		13.0	2.4
Rps. spheroides	Q_{10}	5.3	—		22.8	4.0
Rps. capsulatus	Q_{10}	5.9	—		27.0	4.5
Rps. palustris	Q_{10}	4.5	—		20.0	4.4
Rhodopseudomonas sp. ("FRNY")	Q_{10}	3.6	—		21.2	5.9
Rm. vannielli	Q_{10}	3.0	—		31.0	10.3

* μmoles/g dry weight. All values at steady state (estimated error $\leqslant 5\%$).

** Bacteriochlorophyll for this species has a characteristic absorption maximum (794 mμ) different from the typical bacteriochlorophyll of Athiorhodaceae ($\lambda_{max} = 772$ mμ). Hence no value for $\Delta\varepsilon_{\mu M}$ is available; however, the absorbance at 794 mμ was 560/g dry weight for these bacteria.

AND REDFEARN[6]. For experiments on fresh bacteria, quinones were extracted and assayed by an earlier procedure of REDFEARN[7]. To determine the isoprenoid chain lengths, we used thin-layer chromatography on silica gel G plates, impregnated with 5% paraffin in light petroleum. The chromatograms were developed in the usual manner with the solvent system: acetone–water (95:5, by vol.). As markers, we had available standard samples of Q_6, Q_8, Q_9 and Q_{10}, and the menaquinones MK_2, MK_4, MK_5, MK_8 and MK_9. Q_8 and MK_8 were prepared in pure form from *Rhodopseudomonas gelatinosa* (E. R. REDFEARN, private communication) Q_6 and Q_{10} were purchased from Sigma Chemical Co., St. Louis, Mo. All other preparations were a generous donation from Hoffman-La Roche and Co., Basel, Switzerland.

Results of analyses for stationary phase cells are shown in Table I. All growth experiments were preceded by an anaerobic dark incubation period (usually overnight) which we found completely effective in abolishing the lag period usually observed for Athiorhodaceae in transition from dark aerobic to light anaerobic growth[8]. The stationary phase was usually reached within 20 h for all species, beginning with a 5% inoculum. Results which deserve special comment are:

(1) In all species which contain Q_{10}, MK is absent. However, in the single case of *R. rubrum*, a second component (rhodoquinone[9,10]) is present. This is also true for the G-9 mutant (J. MAROC, previously unpublished). It has been reported[11] that Q_{10} and rhodoquinone in a ratio of approx. 5:1 are bound to *R. rubrum* chromatophores

and that removal of quinones abolishes photophosphorylation activity which can be restored by addition of Q_{10}, but not by rhodoquinone.

(2) In all species which contain ubiquinone other than Q_{10}, a menaquinone with the corresponding isoprenoid side chain is the second (minor) component. This has been noted previously in *Chromatium vinosum*[12], *Escherichia coli*[13] and *Proteus vulgaris*[13], all of which contain Q_8 and MK_8. An especially interesting example is provided by *Euglena gracilis*[14] in which Q_9, PQ_9 and rhodoquinone-9 are found[15].

(3) Large quantities of ubiquinone are found invariably as the major component, even in *Rhodopseudomonas palustris* which has been reported previously to have a much lower ubiquinone content[16].

(4) Lyophilized powders, stored below freezing temperatures and representing an accumulation over long periods of cells from a variety of cultures of the same organisms, give results which differ greatly from those obtained with fresh homogeneous cultures. Thus, a *Rps. palustris* powder sample contained 1.65 μmoles/g dry weight, as compared with 4.5 μmoles/g dry weight for fresh stationary cells (Table I). Moreover, most of the bacteriochlorophyll was degraded in the powder samples.

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