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Ubiquinone Concentrations in Athiorhodaceae Grown under Various Environmental Conditions

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1. The nature and concentration of ubiquinone in six species of Athiorhodaceae have been examined after growth under aerobic and photosynthetic conditions. 2. Increase in ubiquinone concentration during adaptive synthesis of photosynthetic pigments by *Rhodopseudomonas spheroides* incubated under low-aeration conditions was observed. 3. The nature of the carbon source was found to have a marked effect on ubiquinone, as well as bacteriochlorophyll, concentrations.

The characterization of the group of compounds collectively called ubiquinones and having the structure 2,3-dimethoxy-5-methylbenzoquinone with a polyisoprenoid side chain of various lengths at the 6-position of the benzene ring (Morton *et al.* 1958; Wolf *et al.* 1958) led to intensive work to reveal their metabolic role. A possible function was indicated by the concentration of ubiquinone in mitochondria and attention has been directed to their role in electron transport (see Wolstenholme & O'Connor, 1961).

The concentration of ubiquinone in numerous bacteria has been determined (Lester & Crane, 1959; Page *et al.* 1960; Fuller, Smillie, Rigopoulos & Yount, 1961; Bishop, Pandya & King, 1962) and all five naturally occurring ubiquinones have been detected, i.e. Q-6, Q-7, Q-8, Q-9, Q-10, where the number refers to the number of isoprene units in the side chain. In bacterial metabolism ubiquinone has been implicated both in aerobic electron-transfer mechanisms (Kashket & Brodie, 1963) and in photosynthetic phosphorylation (Rudney, 1961). Because of their ability to grow under either aerobic oxidative conditions or anaerobic photosynthetic conditions (van Niel, 1941) the ubiquinone content of Athiorhodaceae (non-sulphur purple bacteria) has been examined in organisms grown in both types of environment and on different carbon sources. Athiorhodaceae grown aerobically contain only traces of the bacteriochlorophyll and carotenoids that are abundant in organisms grown photosynthetically (Cohen-Bazire, Sistrom & Stanier, 1957; Lascelles, 1959). Anaerobic photosynthetic cultures of *Rhodospirillum rubrum* have been shown to contain more ubiquinone than do aerobic cultures (Sugimura & Rudney, 1962; Geller, 1962). *Rhodomicrobium vannielii*, an atypical member of the Athiorhodaceae, is not capable of aerobic growth and is therefore an obligate photoanaerobe (Duchow & Douglas, 1949). It was also studied and its quinone and bacteriochlorophyll concentrations are compared with those of the five typical species examined. A preliminary account of some of the results presented has been published (Carr, 1964).

EXPERIMENTAL

Organisms. *Rhodopseudomonas spheroides* N.I.C.B. no. 8253, *Rhodospirillum capsulata* van Niel, 2.3.11, *Rhodospirillum palustris* van Niel, 2.1.7, *Rhodospirillum gelatinosa* van Niel, 2.2.3, and *Rhodospirillum rubrum* N.I.C.B. no. 8255 were obtained through the courtesy of Dr J. Lascelles, of the Department of Biochemistry, University of Oxford. *Rhodomicrobium vannielii* strain 450 was kindly supplied by Dr H. C. Douglas, of the Department of Microbiology, University of Washington, Seattle, Wash., U.S.A. Stock cultures were maintained as described by Lascelles (1959) except that cultures of *Rm. vannielii* were made completely anaerobic by a few crystals of pyrogallol and a drop of saturated K_2CO_3 solution placed on the surface of the cotton-wool plug, which was forced into the tube by a rubber bung.

Media. All organisms were grown on a medium (Lascelles, 1959) containing (mg./l.): KH_2PO_4, 500; K_2HPO_4, 500; $(NH_4)_2HPO_4$, 800; $MgSO_4,7H_2O$, 200; $CaCl_2$, 40; $MnSO_4,4H_2O$, 4; ferric citrate, 3; nicotinic acid, 1; thiamine hydrochloride, 1; biotin, 0·01. Sodium hydrogen malate (2·7g./l.) and sodium glutamate (1·9g./l.) were the usual carbon sources. The pH was adjusted to 6·9 with 10N-NaOH before autoclaving. With *Rs. palustris* the above medium was supplemented with *p*-aminobenzoic acid (1μM); *Rm. vannielii* does not require vitamins but was grown in the standard medium for convenience.

Growth conditions. Photosynthetic cultures were grown in Roux bottles, or 250ml. reagent bottles filled nearly to the neck; *Rm. vannielii*, which requires strictly anaerobic conditions, was grown in reagent bottles completely filled and sealed with a glass stopper. All photosynthetic cultures

were incubated at 30–32°, 16 in. from a bank of 60 w tungsten bulbs. Aerobic cultures were shaken on a rotary shaker (100 rev./min.) in 250 ml. conical flasks containing 60 ml. of medium at 30–32° without illumination. All cultures were inoculated with 1% of a 24 hr. photosynthetically grown broth culture (Lascelles, 1959) and harvested near the end of their exponential growth phase (approx. 1·0 mg. dry wt./ml.) and washed in half their volume of 0·01 M-potassium phosphate buffer, pH 7·0. The photosynthetically grown cultures were deep brown–red, whereas the aerobic organisms were pale pink.

Isolation of ubiquinone. A suspension of washed bacteria was freeze-dried and the weighed material (100–900 mg.) extracted in a Soxhlet thimble with 500 ml. of iso-octane (2,2,4-trimethylpentane) for 5 hr. Most of the iso-octane was removed by distillation under reduced pressure and the remainder evaporated in a stream of nitrogen. The total lipid extract was dissolved in light petroleum (b.p. 40–60°) and poured on to a 5 g. column of acid-washed partially deactivated alumina. Ubiquinone was eluted with the 6% (v/v) diethyl ether in light petroleum (b.p. 40–60°) fractions (Heaton, Lowe & Morton, 1957).

Determination of ubiquinone. The reduction of the 275 mμ peak of ubiquinone by sodium tetrahydroborate in spectroscopically pure ethanol was used for quantitative determination (Lester & Crane, 1959).

Determination of bacteriochlorophyll. Bacteria were extracted by methanol and bacteriochlorophyll was estimated spectrophotometrically by the method of Cohen-Bazire *et al.* (1957).

Determination of growth. The growth of organisms was determined turbidimetrically in an EEL colorimeter and dry wt./ml. found by comparison with an appropriate calibration curve.

Paper chromatography. The particular ubiquinone isoprenologue in each of the species examined was identified by chromatographic comparison with known ubiquinones (Q-6, Q-7, Q-8, Q-9, Q-10) kindly supplied by Hoffmann–La Roche and Co. Ltd., Basle, Switzerland. A reverse-phase system similar to that of Linn *et al.* (1959) was used in which Whatman no. 1 paper was soaked in 5% (v/v) liquid paraffin in light petroleum (b.p. 40–60°) and allowed to dry at room temperature. After the standard and unknown ubiquinone samples were applied the chromatogram was developed with water–*NN*-dimethylformamide (1:39, v/v). The ascending chromatography took 3–4 hr. to move 25 cm. The positions of the ubiquinone homologues were revealed as dark areas when the chromatogram was examined in ultraviolet light.

Table 1. *Chromatographic behaviour of ubiquinone extracted from Athiorhodaceae*

Samples of ubiquinone extracted from photosynthetically grown organisms were chromatographed on reverse-phase paper together with known ubiquinone isoprenologues (see the Experimental section).

Ubiquinone homologue:	R_F
Q-10	0·17
Q-9	0·29
Q-8	0·39
Q-7	0·51
Q-6	0·61
Ubiquinone extracted from:	
Rs. capsulata, Rs. palustris, Rs. spheroides, Rp. rubrum, Rm. vannielii	0·17
Rs. gelatinosa	0·39

RESULTS

Identification of the ubiquinone homologue. An examination by reverse-phase paper chromatography of ubiquinone extracted from each of six species of Athiorhodaceae and a comparison with five standard ubiquinone homologues are presented in Table 1. With one exception all the organisms examined contained ubiquinone that behaved chromatographically as the decaprenyl ubiquinone

Table 2. *Ubiquinone and bacteriochlorophyll contents of Athiorhodaceae grown aerobically or photosynthetically*

The values stated are the means of several determinations (see the Experimental section), the ranges and numbers of which are given in parentheses.

Organism	Growth conditions	Ubiquinone (mg./g. dry wt.)	Ubiquinone (μmoles/g. dry wt.)	Bacteriochlorophyll (μmoles/g. dry wt.)	Bacteriochlorophyll/ubiquinone ratio
Rs. capsulata	Aerobic	2·27 (2·50–2·17; 4)	2·68	1·05	—
	Photosynthetic	3·55 (4·00–2·95; 4)	4·20	18·0	4·3
Rs. gelatinosa	Aerobic	1·22 (1·40–1·00; 3)	1·71	6·6	—
	Photosynthetic	1·98 (2·40–1·82; 4)	2·78	27·7	10·0
Rs. palustris	Aerobic	0·17 (0·20–0·14; 3)	0·20	0·41	—
	Photosynthetic	0·16 (0·19–0·13; 3)	0·19	14·8	78·0
Rs. spheroides	Aerobic	1·28 (1·40–1·15; 8)	1·51	0·33	—
	Photosynthetic	2·81 (3·60–2·18; 6)	3·32	24·0	7·2
Rp. rubrum	Aerobic	1·11 (1·43–0·70; 5)	1·31	0·83	—
	Photosynthetic	2·13 (2·53–1·92; 4)	2·52	7·30	2·9
Rm. vannielii	Photosynthetic	0·33 (0·43–0·25; 5)	0·39	14·0	36·0

(Q-10). The ubiquinone present in *Rs. gelatinosa* ran with the octaprenyl ubiquinone (Q-8).

Ubiquinone concentrations in aerobic and photosynthetic cultures. The amount of ubiquinone present in the five species examined that were capable of either aerobic or photosynthetic growth varied markedly between species. *Rs. capsulata*, *Rs. gelatinosa*, *Rs. spheroides* and *Rp. rubrum* contained similar amounts of ubiquinone and in each case the amount found in aerobic (non-pigmented) bacteria was approximately half that found in photosynthetically grown organisms (Table 2). The amount of ubiquinone in *Rs. palustris* was about one-tenth of that found in the other species that were capable of aerobic or photosynthetic growth and was the same in organisms from either type of culture. *Rm. vannielii*, unusual among the Athiorhodaceae in that it is incapable of aerobic growth, contained slightly more ubiquinone than *Rs. palustris* but considerably less than the other species examined. The suppression of bacteriochlorophyll synthesis in Athiorhodaceae by aerobic growth is well known, and except in *Rs. palustris* it is accompanied by a much smaller decrease in ubiquinone content. The bacteriochlorophyll/ubiquinone ratio in photosynthetic cultures exhibits considerable species variation (Table 2), from 2·9 in *Rp. rubrum* to 78·0 in *Rs. palustris*. The two very high bacteriochlorophyll/ubiquinone ratios occur in *Rm. vannielii* and *Rs. palustris* and reflect the relatively low concentrations of ubiquinone present in these organisms.

In extracts of both aerobic and photosynthetically grown *Rs. spheroides* over 90% of the ubiquinone was present in the particulate fraction (sedimented at 100000*g* for 90min.), which carried the photosynthetic pigments. Only a trace was recovered in the supernatant fraction, which contained most of the protein present in the extract.

Formation of ubiquinone during the adaptive synthesis of photosynthetic pigments by Rs. spheroides. When an aerobically grown non-pigmented suspension of *Rs. spheroides* is incubated under low-aeration conditions the rapid onset of bacteriochlorophyll and carotenoid formation may be observed (Lascelles, 1959). In such conditions, while the bacterial suspension was synthesizing the photosynthetic pigments and associated proteins (Bull & Lascelles, 1963), the ubiquinone concentration increased by 75%, from the amount found in aerobic cultures to that associated with photosynthetically grown organisms (Fig. 1*a*). A similar result was obtained if, after incubation for 3½hr. under low-aeration conditions, the bacterial suspension was transferred to anaerobic light conditions (Fig. 1*b*). In each case the adaptive synthesis of the photosynthetic pigments was accompanied by a marked increase in ubiquinone content, while the total cell material, measured as dry wt./ml., remained virtually constant.

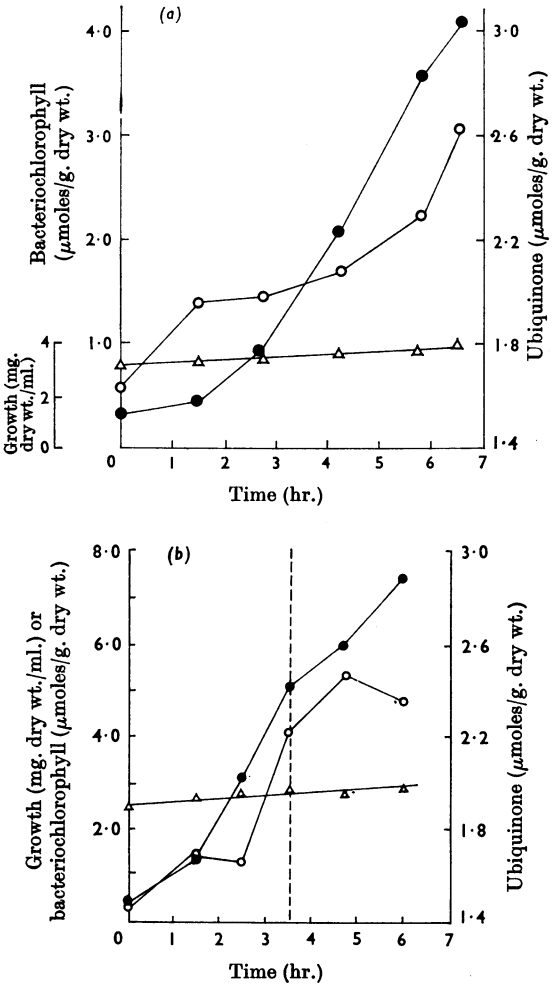

Fig. 1. Adaptive formation of photosynthetic pigments and ubiquinone by *Rs. spheroides* incubated under 'low-aeration conditions'. Analyses were by the methods described in the Experimental section. ○, Ubiquinone; ●, bacteriochlorophyll; △, bacterial growth. 'Low-aeration conditions' were achieved by suspending washed organisms (2–3mg. dry wt./ml.) in fresh growth medium at 31° in a 500ml. measuring cylinder, and aerating with a gentle flow of air (approx. 30ml./min.) from the base of the cylinder. Portions (50ml.) were removed at intervals for analysis. In (*a*) the bacterial suspension was incubated under low-aeration conditions for the duration of the experiment; in (*b*) the suspension was transferred to photosynthetic conditions, as used for growth, after 3½hr. (broken vertical line).

Table 3. *Effects of substrate on ubiquinone and bacteriochlorophyll concentrations*

Organisms were grown under the conditions and on the medium described (see the Experimental section) except that the malate and glutamate in the medium were replaced by the carbon source indicated (10 mM) plus $NaHCO_3$ (20 mM), the latter being added as a sterile solution after the rest of the medium had been autoclaved. The results presented are the means of at least three determinations.

	Growth conditions	Aerobic	Photosynthetic	
Organism	Carbon source	Ubiquinone (μmoles/g. dry wt.)	Ubiquinone (μmoles/g. dry wt.)	Bacteriochlorophyll (μmoles/g. dry wt.)
Rs. spheroides	Acetate	0·47	0·84	2·4
	Succinate	0·61	2·48	5·8
	Malate	1·61	3·30	16·8
Rm. vannielii	Acetate	—	0·17	4·2
	Ethanol	—	0·24	6·3
	Succinate	—	0·18	4·3
	Malate	—	0·63	10·3

Effect of growth substrate on ubiquinone concentration. The effect of various carbon sources on the amount of ubiquinone was examined, after aerobic and photosynthetic growth in *Rs. spheroides* and after anaerobic photosynthetic growth in *Rm. vanniellii*. Organisms grown on acetate plus sodium hydrogen carbonate contained only about one-third of the ubiquinone found in bacteria cultures on malate plus sodium hydrogen carbonate (Table 3). The amount of bacteriochlorophyll formed by photosynthetically grown cultures of *Rs. spheroides* was known to be affected by the nature of the carbon source (Stanier, 1958; Kornberg & Lascelles, 1960). This was confirmed by using the atypical member of the family, *Rm. vannielii*, although the degree of suppression of bacteriochlorophyll by growth on acetate rather than malate was less than that obtained with *Rs. spheroides* (Table 3). Ubiquinone and bacteriochlorophyll concentrations in both organisms examined responded in a similar manner to the carbon sources employed.

DISCUSSION

In the Athiorhodaceae anaerobic photosynthetic growth conditions yielded higher concentrations of ubiquinone than were found in aerobic cultures; this is not the case in non-photosynthetic facultative anaerobes (Bishop *et al.* 1962). Four out of the five species of Athiorhodaceae that were capable of either aerobic or photosynthetic types of growth contained appreciably more ubiquinone after photosynthetic anaerobic culture (Table 2). *Rs. palustris* was exceptional in that it contained similar relatively low concentrations after either type of culture. The twofold difference in *Rs. rubrum* should be compared with a fourfold increase found in this bacterium by Sugimura & Rudney (1962); this discrepancy may be due to species or procedural differences. The atypical *Rm. vannielii* contained relatively low concentrations of ubiquinone but possessed typical amounts of bacteriochlorophyll. The adaptive formation of the photosynthetic apparatus by incubation of non-pigmented organisms under low-aeration conditions has been closely examined. The production of bacteriochlorophyll and carotenoids, enzymes concerned in their synthesis, and associated protein formation and haemoprotein synthesis have been extensively studied (Lascelles, 1959, 1960; Carr & Lascelles, 1961; Bull & Lascelles, 1963; Porra & Lascelles, 1965). Under these conditions ubiquinone was formed concurrently with bacteriochlorophyll (Fig. 1) and enhanced synthesis did not precede the onset of bacteriochlorophyll synthesis.

Rs. gelatinosa, in contrast with the other species examined, contained Q-8 rather than Q-10 (Table 1). This variation between species is more marked in the sulphur purple bacteria (Thiorhodaceae), which contain at least three ubiquinone homologues: *Chromatium* strain D possesses Q-7 (Fuller *et al.* 1961), *Chromatium* 8379 possesses Q-10 (Green, Price & Gare, 1959) and *Chromatium vinosum* possesses Q-8 (Osnitskaya, Threlfall & Goodwin, 1964); the last-named workers have suggested that the particular homologue present may be of value in bacteria systematics.

The marked influence of carbon source on the ubiquinone concentration of *Rs. spheroides* and *Rm. vannielii* is noteworthy, although such an effect on bacteriochlorophyll is already known. The results obtained with aerobically grown *Rs. spheroides* are of interest because they indicate that the mechanism determining ubiquinone content remains open to environmental influence when bacteriochlorophyll formation is considerably

diminished. In contrast, the variation in ubiquinone content of photosynthetically grown organisms is accompanied by similar changes in bacteriochlorophyll content.

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