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THE UBIQUINONE HOMOLOGUE OF THE GREEN MUTANT OF *RHODOPSEUDOMONAS SPHEROIDES**

G. A. PETERS** AND R. A. CELLARIUS

Department of Botany, University of Michigan, Ann Arbor, Mich. 48104 (U.S.A.)

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SUMMARY

Ubiquinone was isolated from aerobically, semi-aerobically, and photosynthetically grown cells of the green mutant of *Rhodopseudomonas spheroides* (strain 2.4.1 Ga). The ubiquinone in each case was characterized by its oxidized and reduced absorption spectra and the specific homologue was identified chromatographically. Under all growth conditions ubiquinone-10 was the only homologue found to be present.

The presence of ubiquinone has been clearly established in members of the Athiorhodaceae, including *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum* and in the Thiorhodaceae, especially in *Chromatium* species[1-15]. Ubiquinone has been shown to be an integral part of both the photosynthetic and oxidative electron transport systems in the Athiorhodaceae. In *R. rubrum* a coupling between the oxidative and photochemical systems has been suggested to occur by reversible electron transfer reactions between quinones of the two systems and by the high energy state ($X \sim I$)[1]. The possibility that the quinones of the two systems are separate in nature, possibly in pools, has been inferred by the observation that the difference spectrum obtained for the reduction of endogenous ubiquinone by light differs from that obtained by chemical reduction with succinate[2]. While recent experiments have indicated that ubiquinone is not the primary electron acceptor in the photosynthetic system[3,4], there is very strong evidence that ubiquinone is part of a highly organized reaction center complex[3,5-7].

Although CARR AND EXELL[8] reported that only ubiquinone-10 was present in aerobically, semi-aerobically, and photosynthetically grown *Rps. spheroides*, strain N.I.C.B. No. 8253, the ubiquinone homologue in *Chromatium* varies from strain to strain[8,10,15] and photosynthetically grown *R. rubrum* has been reported to possess either ubiquinone-9 (refs. 9, 10) or ubiquinone-10 (refs. 8, 11) as well as rhodoquinone[11-14]. Therefore, the purpose of this study was to identify the ubiquinone homologue present in the green mutant of *Rps. spheroides* strain 2.4.1/Ga, derived from A.T.C.C. No. 17023 (*cf.* refs. 16, 17), which we have routinely used for repigmentation studies[18,19], and to determine whether the same or different homologues

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** Present address: Charles F. Kettering Research Laboratory, Yellow Springs, Ohio 45387 (U.S.A.).

were present in cells which were grown under either oxidative or photosynthetic conditions. We postulated that if different homologues were involved in oxidative and photosynthetic electron transport the ratio of one to the other should vary with the conditions of growth and that both should be present when pigment synthesis was induced in totally depigmented cells by transfer to reduced oxygen tension in the dark.

Cells were grown in modified Hutner's medium (see ref. 16) which is equally suitable for aerobic, semi-aerobic, and photosynthetic growth. Photosynthetic cultures were grown at moderate light intensity ($7 \cdot 10^4$ ergs/cm^2 per sec) in 250-ml glass stoppered bottles and harvested prior to the stationary growth phase. Total depigmentation and the growth of semi-aerobic cultures (4 % oxygen) were conducted as described previously[18] with the exception that the culture volume was increased 4-fold and the entire culture was harvested 4 h or 22 h after the transfer to semi-aerobic conditions. Aerobic cultures were obtained in the same manner except growth was continued under vigorous aeration in the light. In each case the cells were harvested by centrifugation, washed with 0.05 M potassium phosphate buffer (pH 6.8) and recentrifuged. Subsequent steps were carried out in minimal light. The cell pellets were extracted twice with acetone–methanol (7:2, v/v). These extracts were transferred to light petroleum (b.p. 30–60°) and partition was facilitated by the addition of an aqueous 10 % NaCl solution. The aqueous acetone–methanol layer was re-extracted with light petroleum and then discarded. The light petroleum fractions were washed several times with 10% NaCl solution and then with distilled water, discarding the aqueous layer in each case after complete separation of the phases. The light petroleum fractions were evaporated to dryness under nitrogen, the residue redissolved in acetone, dried over anhydrous Na_2SO_4, and applied as a band to a Gelman-type SA thin-layer chromatography sheet adjacent to a marker of authentic ubiquinone-10. The chromatogram was developed in a Gelman Thin-Layer Chromatography Chamber with a solvent system consisting of benzene–chloroform (3:1, v/v). Although this solvent system does not separate the various ubiquinone homologues, it does separate the ubiquinone(s) from most other pigmented compounds[20]. The bands which migrated in the region of the authentic ubiquinone-10 were excised, being careful to avoid the marker itself, cut into small strips and eluted into 95 % ethanol. Part of this solution was used to obtain oxidized and reduced spectra on a Unicam SP-800A recording spectrophotometer. The quinone was reduced by adding several grains of $NaBH_4$.

For the chromatographic identification of the ubiquinone homologues the remainder of the samples were evaporated to dryness under nitrogen, redissolved in acetone and chromatographed employing the reversed phase partition chromatographic system of WAGNER *et al.*[21] as modified by BEYER *et al.*[20]. The known ubiquinone homologues were obtained from Dr. Robert E. Beyer. The homologues containing 4, 6, 7, and 9 isoprenoid units were a gift to him from Dr. O. Weiss of Hoffmann–LaRoche and Co., Basle, Switzerland and the ubiquinone-10 from Dr. Arthur R. Wagner of Merck, Sharpe, and Dohme Research Laboratories, Rahway, New Jersey.

A chromatogram of the ubiquinone from each isolation and known ubiquinone homologues is presented in Fig. 1. The extracts from completely depigmented cells, from depigmented cells grown semi-aerobically for 4 and 22 h, and from photo-

synthetically grown cells, each reveal only a single spot which migrated with authentic ubiquinone-10. Oxidized and reduced absorption spectra in 95 % ethanol of the ubiquinones from each isolation were in agreement with that of pure ubiquinone[22]. The characteristic peak for ubiquinone at 275 nm in the oxidized state and 290 nm in the reduced state were obtained in each case. Therefore, in *Rps. spheroides* strain 2.4.1/Ga, as well as in strain N.I.C.B. No. 8253[8], there is only a single homologue, ubiquinone-10, present under clearly defined conditions of aerobic, semi-aerobic, and photosynthetic growth. Since only a single homologue was found, no attempt was made to undertake a quantitative study.

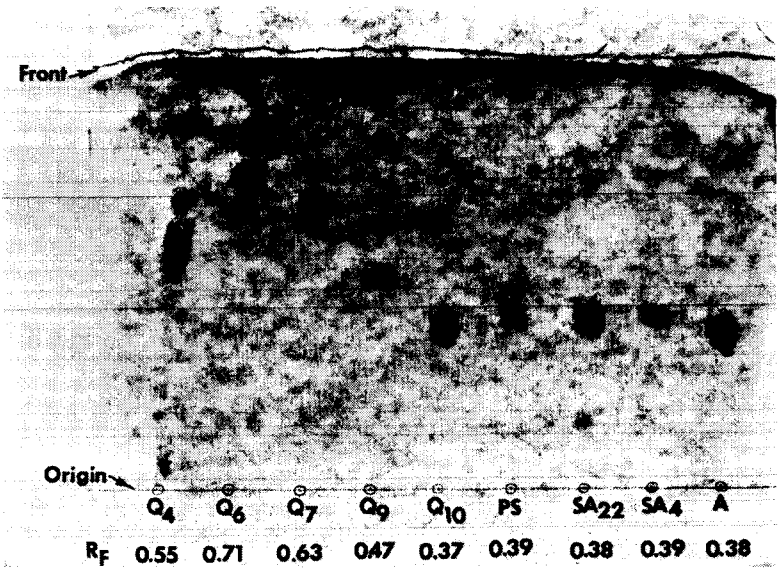

Fig. 1. Reversed-phase thin-layer partition chromatograph of ubiquinone homologues and the ubiquinone isolated from cultures of *Rps. spheroides* (strain 2.4.1/Ga) grown photosynthetically (PS), semi-aerobically for 4 h and 22 h (SA_4 and SA_{22}, respectively) and aerobically until devoid of detectable photosynthetic pigment (A). The developed chromatogram was photographed through a Kodak No. 47 gelatin filter without preliminary staining. The R_F of ubiquinone-4 is consistantly lower than that of ubiquinone-6 in this chromatographic system[20].

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