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THE SOURCE OF OXYGEN IN RHODOPSEUDOMONAS SPHEROIDES CAROTENOID PIGMENT CONVERSION

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THE SOURCE OF OXYGEN IN *RHODOPSEUDOMONAS SPHEROIDES* CAROTENOID PIGMENT CONVERSION

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ABSTRACT

When an anaerobic culture of Rhodopseudomonas spheroides was exposed to O_2^{18}, 94.2% (dark) and 89.6% (light) of the theoretical oxygen incorporation into the pigment spheroidenone was observed. These results are consistent with the conclusion that the sole source of the ketone oxygen in this carotenoid pigment is molecular oxygen.

When anaerobic cultures of Rhodopseudomonas spheroides are exposed to oxygen, the yellow carotenoid pigment spheroidene is rapidly converted to the red carotenoid pigment spheroidenone (Table I). This reaction involves the specific oxidation of the methylene group at carbon 2 to a ketone[1,2].

It has long been considered likely that molecular oxygen contributes one atom to the spheroidenone molecule, but no direct evidence of this was available. A complicating factor was the observation that the rate and extent of the reaction are enhanced by exposure to light[2,3]. The availability of nearly pure O_2^{18} and of improved instrumentation and techniques have made a precise mass spectrographic analysis of the product of this reaction possible. This is the object of this report.

Wild type Rhodopseudomonas spheroides, strain 2.4.1. originally obtained from the collection of Professor C.B. van Niel, was grown anaerobically in the manner described earlier[2] and in amounts sufficient to yield approximately 1 mg of spheroidenone. Pellets of the bacteria, obtained by centrifugation, were resuspended in 10 ml of the modified Hutner's resting cell medium[2], and transferred to 20 ml flasks connected to a Pyrex glass vacuum manifold and evacuated to 20 mm Hg. A gaseous mixture at S.T.P. consisting of 20% O_2^{18} (98.4 atom %)[c] and 80% argon by volume was next introduced into the manifold and the whole system was shaken for three hours at 20°C. One flask was kept in complete darkness while the other was exposed to saturating incandescent illumination during the shaking phase[2]. At the end of this period, the crude pigments were quantitatively extracted according to the method described earlier[2]. Isolation and purification of spheroidenone was obtained by repeated chromatography on 32 x 150 mm columns of Micro-Cel C[d] with 1% acetone in light petroleum. Carrier spheroidenone was prepared by the same procedure.

Spheroidenone 482 mu $E^{1\%}_{1\ cm}$ 2120 (acetone-methanol 7:2).

Samples of approximately 5 mg spheroidenone, including carrier, were pyrolysed with Hg_2Cl_2 at 500°C for an hour, to yield CO_2 according to the method of Rittenberg and Pontecorvo[4]. The O^{18}/O^{16} ratio in CO_2 was determined on a Consolidated Electrodynamics Co. Mass Spectrometer Model 21-130, a cycloid tube type which is capable of good measurements with relatively small samples. Results are shown in Table II. Since there are two oxygen atoms per molecule of spheroidenone, a 100% contribution from molecular oxygen to the ketone would result in a 50% overall incorporation of the isotope in the CO_2. The observed experimental value of 47.1% for the dark culture sample is consistent, within experimental error, with this assumption. Under the conditions of these experiments, synthesis of spheroidene, with incorporation of label into the methoxyl oxygen is improbable. While the incorporation of the label into the light saturated culture sample was lower by 1.5%, this difference is certainly insufficient to suggest that a contribution from oxygen resulting from photolysis of water was involved. On the basis of the evidence presented here, it is most likely that the sole source of the ketone oxygen in spheroidenone is molecular oxygen.

FOOTNOTES

a. This work was done during the tenure of an Advanced Research Fellowship of the American Heart Association.

b. The work described in this paper was sponsored, in part, by the United States Atomic Energy Commission.

c. Molecular Oxygen-18, 98.4 atom % purity, was obtained from the Isotope Division, Weitzman Institute of Science, Rehovot, Israel.

d. Micro-Cel C is a synthetic calcium silicate manufactured by Johns Manville Co. For best results, it was necessary to pyrolyse it in an oven at 400°C for one hour before use.

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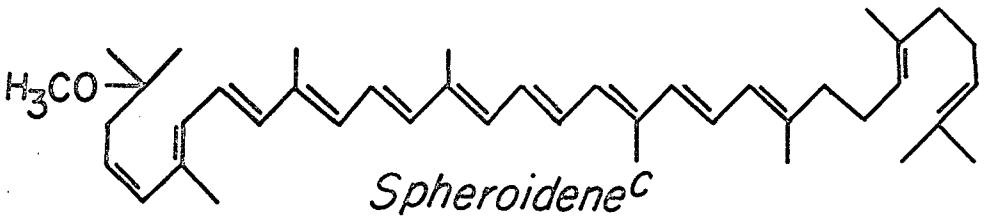
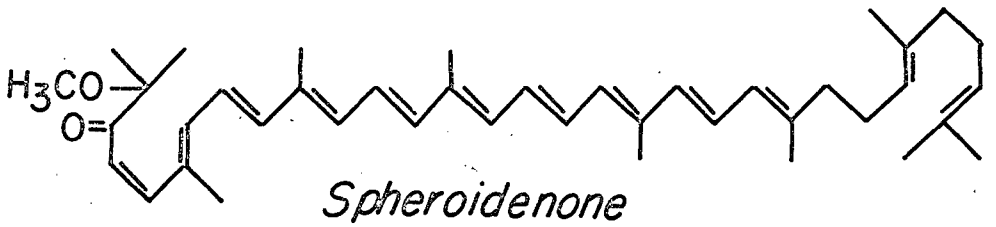
Table I

Structure of Carotenoid Pigments in R. spheroides.



Spheroidene[c]



Spheroidenone

MU-25232

Table II

O^{18} Analysis of Spheroidenone

INCUBATION	ATOM %	ATOM % EXCESS	DILUTION FACTOR Initial*	Carrier	Total	ATOM % EXCESS X DILUTION	% INCORPORATION**
Dark	6.77	6.57	1.64	5.41	7.05	46.3	47.1
Light	8.64	8.44	1.64	3.67	5.31	44.8	45.6

Tank CO_2 control: 0.202 $\pm$ 0.003 atoms per cent O^{18}

* Dilution caused by spheroidenone present before incubation with enriched oxygen.

** Molecular oxygen-18 - 98.2 atoms per cent excess.