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Absolute requirement for oxygen during illumination for photoinduced carotenoid synthesis

A number of nonphotosynthetic microorganisms, such as *Neurospora crassa*[1,2], *Fusarium aquaeductuum*[3] and photochromogenic species of mycobacteria[4–7], produce only traces of carotenoids when grown in the dark. However, when exposed to light for a few minutes and subsequently incubated in the dark, the dark-grown organisms produce substantial amounts of carotenoids *de novo*. The light reaction, like a true photochemical reaction, is independent of temperature, while the dark reactions are temperature-dependent and require O_2 (refs. 4, 7). Recently, RAU[8] has reported that when a suspension of dark-grown *N. crassa* or *F. aquaeductuum* was gassed with N_2 and then illuminated, the organisms produced 10–75 % carotenoids as compared to those that were illuminated in an O_2 atmosphere. This has led RAU[8] to suggest that O_2 does not directly participate in the primary photochemical event but functions as an electron acceptor.

In this paper, we would like to report that in contrast to *F. aquaeductuum* and *N. crassa*, photochromogenic species of mycobacteria, *Mycobacterium marinum* (ATCC 927) and an unclassified species Mycobacterium sp., are not photoinduced unless O_2 is present during illumination (Table I). Our experimental procedure consisted of gassing the bacterial suspension in a suction flask with prepurified N_2 and applying suction at the same time to remove traces of dissolved O_2. Under these strict anaerobic conditions, the bacteria failed to be photoinduced on illumination. However, if bacterial suspensions were only gassed with N_2 and no suction was applied, the bacteria were induced to a certain extent on exposure to light. Apparently, gassing with N_2 alone was not sufficient to remove traces of dissolved O_2. Efforts to use ferricyanide or benzoquinone in place of O_2 for the photochemical reaction were unsuccessful.

TABLE I

ABSOLUTE REQUIREMENT FOR O_2 DURING ILLUMINATION FOR PHOTOINDUCED CAROTENOID SYNTHESIS IN MYCOBACTERIUM SP.

Dark-grown bacteria were washed twice with 20 times their weight of 50 mM phosphate buffer (pH 8.0). After washing, they were suspended in the same buffer so as to obtain a 3.3 % suspension. To 50-ml suction flasks were added, 6-ml aliquots of this suspension, 50 mM glucose and 5 mM $(NH_4)_2SO_4$. These suspensions were gassed with prepurified N_2 for 5 min and simultaneously suction was applied. The bacteria were then illuminated at 0° for 15 min with 700 ft-candles of light. Following illumination, the bacteria were incubated at 30° in air in the dark for 24 h. Bacteria were killed by the addition of 0.6 ml of 40 % trichloroacetic acid and carotenoids were extracted according to the procedure described previously[6].

Treatment	*Carotenoids (μg/100 mg of bacteria dry wt.)*
(1) Dark control	3.2
(2) Illuminated in O_2 atmosphere	45.6
(3) Illuminated under strict anaerobic conditions (suction + N_2 gas)	2.2
(4) Illuminated after gassing with N_2 only (no suction applied)	29.9
(5) Illuminated under strict anaerobic conditions with benzoquinone added	3.2
(6) Illuminated under strict anaerobic conditions with ferricyanide added	3.0

Thus, it appears that O_2 plays a direct obligatory role in the primary photochemical reaction in mycobacteria. We should like to mention that although there is an absolute O_2 requirement during illumination, we have been unable to measure the amount of O_2 used for the photochemical reaction with a Warburg apparatus. This may be due either to a lack of sensitivity of the Warburg apparatus to measure traces of O_2 consumed or that O_2 plays only a catalytic role in the light reaction. Assuming that the first possibility is correct, one may speculate on the role of O_2 during illumination —that of photooxidizing an inhibitory substance or producing a photooxidized compound that may stimulate carotenoid production. We have previously presented evidence that protein synthesis (carotenogenic enzymes) *de novo* precedes actual carotenoid synthesis following exposure to light[6,9]. It is conceivable that light and O_2 act either by photooxidizing a repressor or by producing a photooxidized metabolite which may then induce the synthesis of carotenogenic enzymes[9].

In this connection, it should be noted that light is capable of producing singlet O_2 by photosensitization of certain redox substances (such as flavins and cytochromes which are the possible photoreceptors in mycobacteria[4]) as has been reported by FOOTE AND DENNY[10]. Whether singlet O_2 is produced during the photochemical reaction in mycobacteria (and its possible participation in the photooxidation process) is speculative.

It is not clear why there is an absolute O_2 requirement for the photochemical reaction in mycobacteria but not in *N. crassa* or *F. aquaeductuum*[8]. It is rather unlikely that the mechanism of photoinduction is different. A more plausible explanation is that when the experiment was done to remove O_2 from suspensions of *F. aquaeductuum* and *N. crassa* by gassing with N_2, all traces of dissolved O_2 were not removed prior to illumination. If strict anaerobic conditions had been obtained (for example, by gassing with N_2 and simultaneously applying suction), the possibility is that no photoinduction would have occurred in these organisms.

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