Oxygen Does Not Directly Regulate Carotenoid Biosynthesis in *Rhodopseudomonas capsulata*

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We examined the role of bacteriochlorophyll synthesis on the regulation of carotenoid synthesis in *Rhodopseudomonas capsulata*. Strains capable of making bacteriochlorophyll accumulated greater amounts of carotenoids under low oxygen than they did under high oxygen. However, strains unable to produce bacteriochlorophyll did not regulate their carotenoid production in response to changes in oxygen tension. This indicates that oxygen does not directly regulate carotenoid production.

The studies of Cohen-Bazire et al. (7) demonstrated that oxygen influences the synthesis of both bacteriochlorophyll and carotenoids in members of the family *Rhodospirillaceae*. The introduction of air into an anaerobically growing culture resulted in the complete cessation of bacteriochlorophyll synthesis and drastically reduced carotenoid production. Transcription of the genes for bacteriochlorophyll biosynthesis and for pigment-binding proteins has been shown to be decreased in *Rhodopseudomonas capsulata* by the presence of oxygen (1, 3). Although the mechanism of oxygen control of carotenoid synthesis is unknown, it is known that carotenoid synthesis is greatly reduced by mutations blocking bacteriochlorophyll synthesis (10). Strains of the related bacterium *Rhodopseudomonas sphaeroides* unable to synthesise bacteriochlorophyll also do not synthesise a photosynthetic membrane system (2, 12). The question therefore arises as to whether carotenoid biosynthesis is directly regulated by oxygen or whether it is regulated by some other component of the photosynthetic apparatus, which is itself regulated by oxygen.

This question can be answered by growing various strains with mutations in the bacteriochlorophyll biosynthetic genes under high and low oxygen tensions and determining their specific carotenoid contents. If carotenoid production is in fact not directly regulated by oxygen but is stimulated by the synthesis of bacteriochlorophyll or some other component of the photosynthetic membrane system, then mutants that do not elaborate a photosynthetic membrane system will not regulate carotenoid production in response to changes in oxygen tension. If, however, carotenoid production is directly regulated by oxygen, then *bch* mutants, although having lower specific contents of carotenoids, will still adjust their carotenoid content in response to the oxygen tension.

The *bch* mutants used in this study are all Mu d1(Ap', lac) insertion derivatives of PAS100 (1) and carry the *crtD233* allele (Table 1). Instead of the normal carotenoids, these strains produce neurosporene, hydroxyneurosporene, and methoxyneurosporene (11). These carotenoids have identical spectra and are thus easier to quantify than the normal carotenoids (7). A *crtD233* derivative of PAS100 was obtained by mating the R-factor pRPS404 (9) into PAS100, selecting for kanamycin and streptomycin resistance, and isolating a yellow colony. The resulting strain, AJB514, and a *bchC* mutant, AJB499, were grown in culture tubes containing 10 ml of RCV* medium as described earlier (1). Seven tubes of each strain were inoculated into an initial Klett value of 15 to 20. The cultures were sparged with an air-oxygen mixture so that the dissolved oxygen concentration was 23% and grown to a density of 50 Klett units. At this point, the gas mixture was switched to 92% nitrogen-5% carbon dioxide-3% oxygen. The cultures were grown under low oxygen to a density of 100 Klett units. Cultures were harvested at seven different densities. The carotenoids were extracted with methanol and the absorbance at 468 nm was determined, using an E1% value of 2,320 (7). Figure 1 depicts the results of these experiments. The *bch* strain accumulated very little carotenoid when grown in 23% oxygen. However, when the oxygen tension was reduced to 3%, there was a dramatic increase in the carotenoid content of the culture. When strain AJB499, which has a *bchC* lesion, was grown under a high oxygen tension, it accumulated very little carotenoid. When the oxygen tension was shifted to 3%, there was only a very small increase in carotenoid accumulation. These results indicated that bacteriochlorophyll synthesis is required for the typical carotenoid response to changes in oxygen tension. To confirm this observation, the parental strain AJB514 and four *bch* mutants were grown in RCV* and sparged with either the air-oxygen mixture or the nitrogen-carbon dioxide-oxygen mixture. When the cell density reached 100 Klett units, the carotenoids were extracted as detailed above. The protein pellet was resuspended in 0.2 N sodium hydroxide, and the protein content was determined by the method of Lowry et al. (8).

The specific carotenoid content of these strains under high and low oxygen tensions is shown in Table 2. Each strain

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
</tr>
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<tbody>
<tr>
<td>AJB463</td>
<td>φ(bchH⋅lacZ')700 (crtD233) hsd-1 str-2</td>
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<tr>
<td>AJB478</td>
<td>φ(bchB⋅lacZ')708 (crtD233) hsd-1 str-2</td>
<td>1</td>
</tr>
<tr>
<td>AJB499</td>
<td>φ(bchC⋅lacZ')710 (crtD233) hsd-1 str-2</td>
<td>1</td>
</tr>
<tr>
<td>AJB500</td>
<td>φ(bchG⋅lacZ')711 (crtD233) hsd-1 str-2</td>
<td>1</td>
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<tr>
<td>AJB514</td>
<td>(crtD233) hsd-1 str-2</td>
<td>This study</td>
</tr>
<tr>
<td>PAS100</td>
<td>hsd-1 str-2</td>
<td>13</td>
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</tbody>
</table>

TABLE 1. *R. capsulata* strains

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was grown and assayed three to six times. The carotenoid content of strain AJB514 increased 2.6-fold in response to the lowering of the oxygen tension, whereas the bch strains responded only very slightly to the change in oxygen tension. This indicates that completely functional bacteriochlorophyll is necessary to allow the specific carotenoid content of *R. capsulata* to respond normally to oxygen. Even strain AJB500, which can make the final bacteriochlorophyll precursor bacteriochlorophyllide a, shows greatly diminished regulation of carotenoid.

These experiments, although showing that bacteriochlorophyll production is necessary for normal regulation of carotenoid levels, do not indicate whether the lack of regulation in *bch* mutants is due to the lack of bacteriochlorophyll per se or whether it is due to the inability of *bch* mutants to produce an intracytoplasmic membrane system (2). The slight increase in carotenoid accumulation in the *bchC* mutant AJB499 (Fig. 1) may indicate that carotenoid accumulation is regulated by some component of the photosynthetic apparatus other than bacteriochlorophyll. We are currently testing this possibility by measuring carotenoid levels in mutants lacking one or more of the intracytoplasmic membrane pigment-protein complexes.

Carotenoids play two important roles in photosynthetic bacteria. They serve both to harvest light energy (4) and to protect the cell from photooxidative damage (5). As these pigments are normally associated with proteins in the reaction center and light-harvesting complexes (6), it would seem logical that the synthesis of carotenoids should be coordinated with the synthesis of the other components of the photosynthetic membrane system. Two ways that this coordination might occur are: (i) direct regulation of carotenoid synthesis by the same environmental factors that control the other components of the photosynthetic membrane system, e.g., oxygen, or (ii) regulation of carotenoid synthesis by one of the components of the photosynthetic membrane system. The data presented here suggests that carotenoid synthesis is not directly regulated by oxygen, but instead is controlled by the production of either bacteriochlorophyll or some other component of the photosynthetic membrane system.

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**LITERATURE CITED**


