RHODOPSEUDOMONAS SPHEROIDES: HIGH CATALASE AND BLUE-GREEN DOUBLE MUTANTS

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Received July 18, 1960

Catalase synthesis is induced by aeration in Rhodopseudomonas spheroides (Clayton, 1959a; 1960a,b,c). As a result the catalase content rises from 0.002% of the total protein in uninduced cells (grown anaerobically in the light) to about 1% of the total protein in maximally induced cells. In the course of studying this case of enzyme induction, and of exploring the function of catalase in R. spheroides, it became desirable to obtain a constitutive high-catalase mutant. This was accomplished by growing the organism in a continuous culture and exposing the cells, at 3-day intervals, to 0.1 M H_2O_2. After 3 such exposures, we isolated from the culture a mutant that is rich in catalase regardless of the oxygen tension during growth. The H_2O_2 probably acted both as mutagen and selector.

In this mutant (designated CC1), grown anaerobically in the light, the catalase content is about 5% of the total protein. In cells of the mutant grown aerobically in darkness, catalase constitutes as much as 25% of the protein. This is illustrated by the following example: From 420 mg dry cell mass (about half of which is protein) of the mutant, grown aerobically on agar, 38 mg of purified catalase was obtained. During purification the loss in total enzyme activity paralleled the known mechanical losses; on this basis the starting material contained 54 mg of catalase. The purified enzyme had an absorption spectrum and specific activity appropriate for bacterial catalase (Herbert and

* Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

Pinsent, 1948; Clayton, 1959b); it appeared as a single peak in the ultracentrifuge. With diethylaminoethyl cellulose chromatography and also with starch gel electrophoresis a major and a minor component could be distinguished, in a ratio of about 20:1. The two components have approximately the same specific activity. A detailed characterization of this system, including immunochemical analysis, is in progress.

Griffiths and Stanier (1956) have described photo-oxidative killing of a blue-green (carotenoidless) mutant of R. spheroides. Catalase, by destroying H_2O_2, might modify this killing. For this reason we have sought mutants of R. spheroides that combine the traits of high catalase content and carotenoid deficiency. Three such mutants, two blue-green and one green, have been obtained from mutant strain CC1 by exposure to ultraviolet, by the procedure of Griffiths and Stanier (1956). The catalase content of these double mutants is the same as that of the parent high-catalase mutant.

The green high-catalase phenotype (double mutant strain CC1 R8) accumulates carotenoids of the neurosporene group instead of the normal yellow and red carotenoids. Of the two blue-green high-catalase mutants, one (CC1 R22) accumulates phytoene and phytofluene; the other (CC1 R26) accumulates neither of these nor any related compound absorbing light in the range 200-600 mμ. The blue-green mutant of Griffiths and Stanier (1956) accumulates only phytoene. The existence of these new phenotypes supports the biosynthetic pathway suggested by Zechmeister and Pinckard (1948) and elaborated most recently by Jensen et al. (1958).

Preliminary experiments indicate that a high catalase content affords no protection against photo-oxidative killing of the blue-green mutants of R. spheroides, nor does it protect against killing of the normally pigmented form by 250-kv X rays. These experiments do not support the role of free H_2O_2 as a lethal intermediate in either of these killing processes, in R. spheroides.

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