Protein Synthesis in the Induced Formation of Catalase in Rhodopseudomonas spheroides

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Rhodopseudomonas spheroides is a facultative photoheterotroph in which catalase synthesis can be induced by aeration (1). In cultures grown anaerobically in the light, catalase constitutes only about 0.005% of the dry cell mass. In contrast, the catalase content of cultures grown aerobically is as high as 0.3%. If an illuminated, anaerobic culture is exposed to air, a vigorous synthesis of catalase ensues. Under these conditions the utilization of oxygen is slight; the energy for growth is provided mainly through photosynthesis (1).

In terms of the range of catalase content attainable, R. spheroides is superior to other microbes in which inducible catalase has been observed. The catalase of R. spheroides is easily purified and has been characterized extensively (2); its assay in intact cells is simple and accurate (1). The metabolic versatility of R. spheroides makes it possible to study induced catalase synthesis under a variety of environmental conditions. For these reasons the present system holds promise for the study of induced enzyme synthesis and of the function of catalase. Further characterization of this system is therefore desirable.

Induced synthesis of catalase in R. spheroides requires sources of carbon, nitrogen, and energy (1). Inhibitors, such as tetracycline, chloramphenicol, and ultraviolet irradiation, exert parallel effects on growth and induced catalase synthesis (1). These results suggest that the protein of the newly formed catalase is synthesized de novo. This paper describes radiocarbon-incorporation experiments that support this conclusion. Similar experiments by Hogness et al. (3) and by Rotman and Spiegelman (4) have shown that, in the induced synthesis of β-galactosidase in Escherichia coli, the enzyme-protein is synthesized de novo.

EXPERIMENTAL

R. spheroides, strain 2.4.1, was grown in a chemically defined medium (2) containing growth factors, minerals, malate, glutamate, and acetate. Illuminated, anaerobic cultures were grown in completely filled glass-stoppered 500-ml bottles, at 30°, about 20 inches from two 750-watt tungsten lamps. Growth of such cultures became limited through exhaustion of organic sources of carbon; the final catalase content had the low value (about 0.002% of the dry cell mass) characteristic of uninduced cells. Each mature 500-ml culture was centrifuged, and the cells were resuspended in 2500 ml of fresh growth medium containing 0.02 M ammonium malate but lacking glutamate and acetate. 1

Induced catalase synthesis was made to proceed rapidly in the foregoing suspensions by aerating them gently under strong illumination. The suspensions were held in 2500-ml cylinders placed 20 inches from two 750-watt lamps and surrounded by mirrors. After several hours of aeration, the catalase content of the cells was about 0.02 to 0.08% of their dry mass.

C14 incorporation was studied in two ways. In Experiment I the cultures were grown and resuspended in unlabeled media. At the start of aeration and every hour thereafter, 2.5 mmoles of NaHCO3, containing 40 μc of C14, were added to each 2500 ml of suspension. After 4 hours, 10 liters of suspension were harvested; the remaining 5 liters were harvested after 11 hours. These samples were treated as described later, to yield purified catalase fractions and a noncatalase protein fraction. Radioactivity of these fractions could then be assayed.

In Experiment II the cultures were grown in the presence of acetate-2-C14 (0.2 μc per liter); the cells were then washed and resuspended in unlabeled medium. These low catalase cells, containing C14-labeled protein, were incubated in the dark for 30 minutes before aeration in the light, to reduce the endogenous pool of labeled carbon compounds. Induced synthesis of catalase was then allowed to proceed in the unlabeled medium; 600 ml of suspension were harvested initially and 9600 ml after 5 hours. These samples, again, were fractionated as described.

Purification of catalase was performed as described earlier (2). The third step of the purification procedure, CHCl3 denaturation, yielded a catalase-free protein fraction, Fr, which was washed four times with acetone. The final step, chromatography on diethylaminoethyl cellulose, yielded two protein fractions. One, C+, contained about 50% catalase; the other, C-, about 20%.

Radioactivity was assayed by converting the carbon in each of these fractions to CO2 by wet combustion (5) and precipitating it as BaCO3. This was collected on a planchet, weighed, and counted in an end window Geiger-Müller counter. Corrections for self-absorption were applied.

Catalase was assayed by iodometric titration; cell mass was estimated turbidimetrically (1).

RESULTS AND DISCUSSION

The results of Experiments I and II are shown in Table I. Growth and catalase synthesis during the experiments are tabulated under "cell mass" and "catalase." Note that the cell mass did not increase during the 5 hours of Experiment II. Some turnover of cell material during this experiment is indicated by the slight decline in specific activity of Fr.

Analysis of these experiments requires that the specific activity of the pure catalase be computed. If this is denoted Ap, and the specific activity of "general" protein is denoted Ag, then in
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TABLE I

C14-Labeling of catalase and general protein during induced synthesis of catalase in R. spheroides

Cells were grown anaerobically in the light and transferred to fresh growth medium. Aeration of these cells, starting at 0 hours, induced the synthesis of catalase. Samples withdrawn at the times indicated were assayed for cell mass and catalase content and for the specific radioactivities of fractions obtained from the cells. C+ and C− are purified catalase fractions; Pr is chloroform-denatured protein.

<table>
<thead>
<tr>
<th>Time (hr.)</th>
<th>Cell mass T/C</th>
<th>Catalase</th>
<th>Fraction Pr</th>
<th>Fraction C+</th>
<th>Fraction C−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Catalyst</td>
<td>S.A.*</td>
<td>Catalyst</td>
</tr>
<tr>
<td>0</td>
<td>0.45</td>
<td>0.015</td>
<td>0.12</td>
<td>54</td>
<td>0.57</td>
</tr>
<tr>
<td>4</td>
<td>0.50</td>
<td>0.125</td>
<td>0.12</td>
<td>54</td>
<td>0.57</td>
</tr>
<tr>
<td>11</td>
<td>1.03</td>
<td>0.155</td>
<td>0.12</td>
<td>46</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Experiment II: Cells grown in medium containing C14-labeled acetate; catalase induced in unlabeled medium

0 | 0.34 | 0.0055 | 0 | 0.98 |
5 | 0.34 | 0.243  | 0 | 0.95 | 61 | 0.42 |

* Specific activity; µe per 25 mg of carbon in Experiment I and µe per 10 mg of carbon in Experiment II.

TABLE II

Specific activities of catalase and general protein in R. spheroides, calculated from data of Table I

See Table I for a description of Experiments I and II. A, and A, denote specific activities of catalase and general protein, respectively. The units are µe per 25 mg of carbon in Experiment I and µe per 10 mg of carbon in Experiment II.

<table>
<thead>
<tr>
<th></th>
<th>Experiment I</th>
<th>Experiment II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (hr.)</td>
<td>0 hr.</td>
<td>4 hr.</td>
</tr>
<tr>
<td></td>
<td>0.96</td>
<td>0.85</td>
</tr>
</tbody>
</table>

A, can be taken as the specific activity of Fraction Pr. Alternatively, Equation 1 can be applied to Fractions C+ and C−, yielding two simultaneous equations that can be solved for A, and A,.

\[
A_{\text{obs.}} = A_c(\% \text{ catalase}) + A_p(100 - \% \text{ catalase})
\]  

(1)

A, can be taken as the specific activity of Fraction Pr. Alternatively, Equation 1 can be applied to Fractions C+ and C−, yielding two simultaneous equations that can be solved for A, and A,.

\[
A_e = \alpha \frac{\text{new catalase}}{\text{total catalase}}
\]  

(2)

and

\[
A_p = \alpha \frac{\text{new protein}}{\text{total protein}}
\]  

(3)

where \(\alpha\) is a constant.

In Fig. 1 the quantities appearing in Equations 2 and 3, computed from the data of Experiment I, are plotted against time. The ratio of new protein to total protein has been replaced by the ratio of new cell mass to total cell mass, since the latter and not the former was measured. It is assumed that ratios of cell

![Fig. 1. Specific activity of catalase compared with the proportion of newly formed catalase in R. spheroides; similar data for general protein.](http://www.jbc.org/)

TABLE III

Specific radioactivity of catalase compared with proportion of initial catalase in R. spheroides and similar data for general protein

Conditions are those of Experiment II (Table I). A, and A, are the specific activities (µe per 10 mg of carbon) of catalase and general protein, respectively. Catalase induced in C14-labeled cells suspended in unlabeled medium.

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Final (5 hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A,</td>
<td>(1.0)*</td>
<td>0.08</td>
</tr>
<tr>
<td>Initial catalase</td>
<td>1.00</td>
<td>0.02</td>
</tr>
<tr>
<td>Total catalase</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>A,</td>
<td>0.98</td>
<td>0.95</td>
</tr>
<tr>
<td>Initial cell mass</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Total cell mass</td>
<td></td>
<td>1.00</td>
</tr>
</tbody>
</table>

* The initial A, was not measured; it probably was equal, within a few %, to the initial A,.
mass and ratios of protein were approximately equal. Fig. 1 shows that Equations 2 and 3 are verified, and that the induced catalase-protein is therefore synthesized *de novo*.

Experiment II was performed with labeled cells suspended in unlabeled medium. If catalase and general protein were synthesized *de novo*, their specific activities should have declined as the labeled starting material was diluted with unlabeled new material. One should then expect that at any time,

\[ A_c = \beta \frac{\text{initial catalase}}{\text{total catalase}} \]  

(4)

and

\[ A_p = \beta \frac{\text{initial protein}}{\text{total protein}} \]  

(5)

where \( \beta \) is a constant. In Table III the quantities appearing in Equations 4 and 5 (with ratios of protein replaced by ratios of cell mass) are compared. \( A_c \) declined sharply during the experiment, corresponding to the extensive dilution of the initial labeled catalase with new unlabeled catalase. In contrast, \( A_p \) declined very little. Thus a synthesis *de novo* of catalase protein is again demonstrated. The final value of \( A_c \) (0.08) was not quite so low as that predicted by the dilution factor (initial catalase/total catalase, 0.02); this probably means that some catalase was synthesized from labeled endogenous reserves.

**SUMMARY**

*Rhodo* pseudomonas *spheroides* synthesizes only minute amounts of catalase when the organism is grown anaerobically in the light. Rapid synthesis of catalase can be induced in an illuminated, anaerobic culture by exposure to gentle aeration. CM incorporation experiments show that, in this induced synthesis, the protein of catalase is formed *de novo*.

**REFERENCES**

Protein Synthesis in the Induced Formation of Catalase in *Rhodopseudomonas spheroides*

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