Kinetics of H_2O_2 destruction in *Rhodopseudomonas spheroides*: Roles of catalase and other enzymes

Synthesis of catalase is induced in *Rhodopseudomonas spheroides* by aeration[1]. In studying the kinetics of this phenomenon, using air or H_2O_2 as inducer, it has become necessary to know the intracellular concentration of H_2O_2 in a variety of experimental situations. This will depend on the action of catalase and other agents that decompose H_2O_2. At concentrations greater than 1 mM, H_2O_2 is destroyed almost exclusively by catalase* in *Rps. spheroides*[2]. It will be shown here that a peroxidase-like activity is important when the concentration of H_2O_2 is less than about 100 μM.

For these experiments, *Rps. spheroides*, strain 2.4.1, was grown anaerobically in the light, as described earlier[1]. Cultures were tested during exponential growth and in the stationary phase. In the range 1–20 mM, the destruction of H_2O_2 by *Rps. spheroides* was assayed by HERBERT's iodometric titration method[4]. This method could be applied to H_2O_2 concentrations as low as 30 μM by increasing the volumes of reagents 10-fold and using more dilute thiosulfate in the final titration. In the range 3–100 μM, H_2O_2 was assayed by its photochemical reaction with luminol, as described by DOLIN[5]. The sensitivity of each method was limited by the interfering effects of dense cell suspensions.

The rate of destruction of H_2O_2 by *Rps. spheroides* depends on the total activity of all peroxide-destroying agents and on the rate at which H_2O_2 can enter the cells. Permeation of H_2O_2 becomes rate-limiting in cells of very high catalase content; the over-all reaction with *Rps. spheroides* then obeys first-order kinetics[2] described by equation (1)

$$-\frac{d[H_2O_2]}{dt} = 0.13\varrho[H_2O_2] \qquad (1)$$

where ϱ is the cell-suspension density in mg (dry cell mass)/ml and t is expressed in seconds. If the rate of peroxide disappearance ($-d[H_2O_2]/dt$) is observed to be much less than 0.13 ϱ $[H_2O_2]$, it can be assumed that intracellular reactions, and not permeation, are rate limiting. Extracellular and intracellular peroxide concentrations are then nearly equal.

The permeability problem can be approached by treating the cells with toluene; this treatment destroys permeability barriers toward H_2O_2 without changing the catalase activity. Such cells could not be used in the luminol assay, however, because they produced a large and variable "blank" signal. The luminol assay was therefore restricted to cases in which $-d[H_2O_2]/dt$ was less than 0.04 ϱ $[H_2O_2]$("permeation error" less than about 25 %).

Fig. 1 shows the rate of disappearance of H_2O_2 as a function of its concentration in the presence of *Rps. spheroides* taken from a culture in exponential growth. The ordinate has been normalized to a cell suspension density of 1 mg/ml. Curve A, for peroxide concentrations up to 10 mM, has the first-order form appropriate to the action of catalase[3]: $-d[H_2O_2]/dt = k'_1 e[H_2O_2]$, where e is the concentration of catalase and k'_1 is a rate constant. In this range of peroxide concentrations the effect of agents other than catalase is nil. Curve B ($[H_2O_2] < 300$ μM) is described accurately by equation (2)

* The catalase of *Rps. spheroides* has been purified; its molecular weight, specific activity, and absorption spectrum have been determined[3].

$$-\frac{d[H_2O_2]}{dt} = k'_1e[H_2O_2] + \frac{V[H_2O_2]}{K_m + [H_2O_2]} \quad (2)$$

in this range the contribution of catalase ($k'_1e[H_2O_2]$) is shown separately by the dashed line. The term $V[H_2O_2]/(K_m + [H_2O_2])$ is an expression of the MICHAELIS-MENTEN kinetics displayed by most enzymes, including peroxidases. For stationary-phase cells the results are the same except for the magnitudes of k'_1e and V. Values

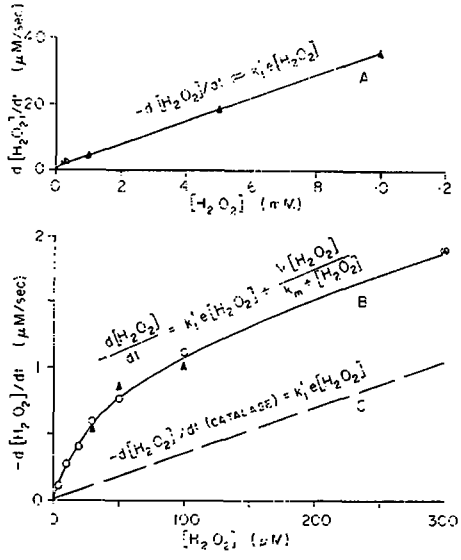

Fig. 1. Kinetics of H_2O_2 destruction by *Rps. spheroides* taken from a culture in exponential anaerobic growth. The ordinate is normalized to a cell-suspension density of 1 mg (dry cell mass)/ml. Curve B is a graph of Equation (2), with $k'_1e = 0.0035$ sec^{-1}, $V = 0.91$ μM sec^{-1}, and $K_m = 28$ μM. The term $k'_1e[H_2O_2]$ represents catalase activity, plotted separately as Curve C. The other term, $V[H_2O_2]/(K_m + [H_2O_2])$, represents a peroxidase-like activity. When H_2O_2 exceeds 1 mM this second term is much smaller than the first ("peroxidase" activity $\ll$ catalase activity). Equation (2) is then approximated by $-d[H_2O_2]/dt = k'_1e[H_2O_2]$ and has the form of Curve A (*cf.* Curve C). ▲, H_2O_2 assayed iodometrically; ○, by the luminol method.

of k'_1e, V, and K_m are shown for growing and stationary-phase cells (suspension density, 1 mg/ml) in Table I. For *Rps. spheroides* catalase, $k'_1 = 53$ μM^{-1} sec^{-1} and the molecular weight is 230,000 (see ref. 3). The values in Table I for k'_1e then give a catalase content of 0.0015 % of the dry cell mass for cells in exponential growth and 0.0026% for stationary-phase cells. At very low peroxide concentrations ($[H_2O_2] \ll K_m$) Equation (2) becomes

$$-\frac{d[H_2O_2]}{dt} = \left(k'_1 + \frac{V}{K_m}\right)[H_2O_2],$$

and for cells in exponential growth the "peroxidase" activity is nearly 10 times as great as the catalase activity ($V/K_m = 0.032$ sec^{-1}, as compared with 0.0035 sec^{-1} for k'_1e). For stationary-phase cells, with $[H_2O_2] \ll K_m$, "peroxidase" activity is about 3 times as great as the catalase activity ($V/K_m = 0.020$ sec^{-1} and $k'_1 = 0.006$ sec^{-1}).

TABLE I

CONSTANTS IN EQUATION (2) DESCRIBING THE DESTRUCTION OF H_2O_2 BY *Rps. spheroides*

Cell-suspension density, 1 mg (dry mass)/ml.

	k'_1 (sec^{-1})	V (μM sec^{-1})	K_m (μM)
Cells in exponential growth	0.0035	0.91	28
Stationary-phase cells	0.006	0.56	28

All the H_2O_2-destroying activity was abolished by boiling the cells for 3 min. Catalase and "peroxidase" activities could be separated cleanly by treatment with toluene and sodium azide, respectively. Exposure to toluene abolished the "peroxidase" activity without affecting the catalase, whereas 10^{-4} *M* azide at pH 7 inactivated the catalase without influencing the "peroxidase" activity. The latter was inhibited 32 % by 10^{-3} *M* azide and 83 % by 10^{-2} *M* azide; these values are appropriate for peroxidases containing heme[6]. A trichloroacetic acid extract of *Rps. spheroides*, prepared as described by VERNON[7], showed negligible peroxidase activity with guiacol and cytochrome *c* as hydrogen donors. Aeration or addition of H_2O_2, sufficient to induce a 10-fold increase in catalase content during 1 h, did not alter the "peroxidase" activity of the initial cells. Thus it is possible to induce wide variations in the relative magnitudes of catalase and "peroxidase" activities in *Rps. spheroides*.

In studies of the effects of H_2O_2 (*e.g.*, in radiobiology), catalase is generally assayed by methods in which the H_2O_2 concentration is millimolar or higher. The destruction of H_2O_2 at much lower concentrations is then predicted from this assay. Such an extrapolation, from the kinetics of peroxide destruction at high concentrations of H_2O_2 to the kinetics at low concentrations, is not justifiable. With *Rps. spheroides*, and perhaps with many other systems, the extrapolation would be grossly in error.

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Obtention du substrat du lysozyme contenu dans la paroi ectoplasmique d'*Eberthella typhi*

Nous avons précédemment montré[1] que la paroi ectoplasmique de *Salmonella* pathogènes pour l'homme, contient un substrat du lysozyme, qui parait—au moins partiellement—responsable de sa rigidité puisque l'activité de l'enzyme se manifeste par sa totale dissolution. Ce substrat, dont on a pu penser qu'il pouvait s'identifier avec l'antigène somatique[2], n'est accessible au lysozyme que si les bactéries ont subi au préalable un traitement approprié dont l'action paraît être d'arracher des constituants