HYDROGEN DONORS AND PEROXIDE CONCENTRATION IN A RESPIRING CELL CONTAINING CATALASE

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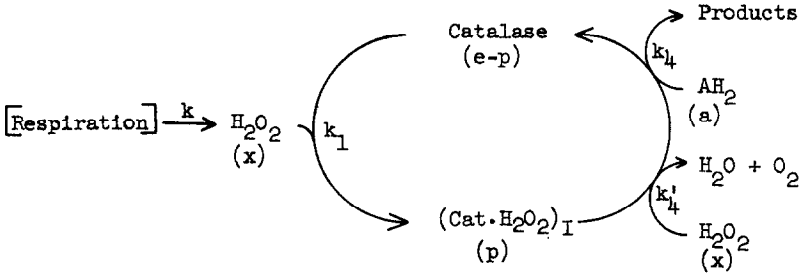
The presence of the active catalase-hydrogen peroxide complex, $(Cat \cdot H_2O_2)_I$, has been demonstrated in aerated Micrococcus lysodeikticus by Chance (1952a). Peroxidatic hydrogen donors such as nitrite, formate, and alcohols were shown to reduce the steady-state concentration of $(Cat \cdot H_2O_2)_I$.

A regulatory function was proposed by Chance for these H-donors on the basis that they depress the intracellular concentration of free H_2O_2. This action was also invoked to explain the protection afforded by nitrite, formate, and alcohols against radiation damage.

It will be shown here that peroxidatic H-donors should increase the concentration of H_2O_2 in respiring cells, rather than lowering it, if the destruction of H_2O_2 is mediated principally by catalase.

The metabolism of H_2O_2 in a respiring cell containing catalase can be represented as follows (Chance and Herbert, 1950; Chance, 1952b):



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In this scheme H_2O_2 is generated at a constant rate k through respiratory activity and destroyed through catalatic and peroxidatic reactions with catalase (AH_2 represents a peroxidatic H-donor). The concentrations of reactants are denoted by the letters in parentheses; e is the total (free plus bound) catalase concentration. Reaction rate constants are denoted k_1, k_4', and k_4, as shown. The corresponding reaction kinetics are described by the equations

$$\frac{dx}{dt} = k - k_1(e-p)x - k_4'px \qquad (1)$$

and

$$\frac{dp}{dt} = k_1(e-p)x - k_4'px - k_4pa \qquad (2)$$

In the steady state, with a reservoir of AH_2 so large that "a" is practically constant, $\frac{dx}{dt} = \frac{dp}{dt} = 0$.

Equations (1) and (2) then yield

$$\left(\frac{k_1e}{k}\right)x = \left[1 + \frac{p}{e}\left(\frac{k_4'}{k_1} - 1\right)\right]^{-1} \qquad (3)$$

and

$$\left(\frac{k_4e}{k}\right)a = \left[1 + \frac{p}{e}\left(\frac{k_4'}{k_1} - 1\right)\right]^{-1}\left(\frac{e}{p} - \frac{k_4'}{k_1} - 1\right) \qquad (4)$$

From these equations one can compute the influence of H-donor concentration (a) on the concentrations of H_2O_2 (x) and of catalase-peroxide complex (p). These relations are shown in Fig. 1, where $\left(\frac{k_1e}{k}\right)x$ and p/e are plotted against $\left(\frac{k_4e}{k}\right)a$ for different values of k_4'/k_1.

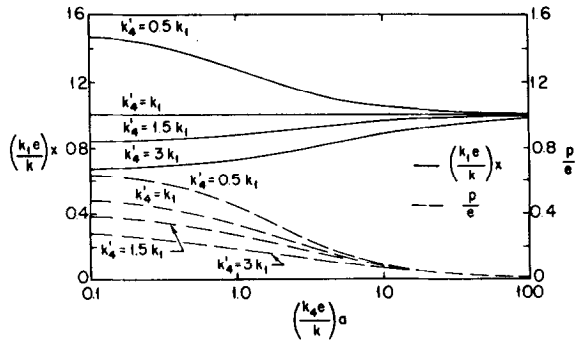

Fig. 1

In every case the concentration of enzyme-substrate compound, p, approaches zero as the concentration of H-donor becomes very large. In

contrast, H_2O_2 concentration rises or falls with increasing H-donor concentration depending on whether k_4'/k_1 is greater or less than unity. This is because the H-donor influences the kinetics in two distinct ways. By accelerating the turnover of enzyme, the H-donor augments the rate of the first reaction with H_2O_2 (H_2O_2 + catalase $\rightarrow$ Cat·H_2O_2). By competing with H_2O_2 in the second step, the H-donor retards the destruction of H_2O_2. These effects just balance when $k_4' = k_1$; the H_2O_2 concentration in the steady state is then independent of H-donor concentration.

The curve for $k_4' = 3k_1$ is appropriate for blood catalase (Chance, 1952b). The one for $k_4' = 1.5k_1$ pertains to the catalases of M. lysodeikticus (Chance, 1952b) and Rhodopseudomonas spheroides (Clayton, 1959). It can be seen that the greatest possible effect of a peroxidatic H-donor on the steady-state concentration of H_2O_2 is to increase it by about 20% (bacterial catalase) or 35% (blood catalase). A catalase for which $k_4' < k_1$ has not been found. Thus no catalase is known for which peroxidatic H-donors will accelerate the destruction of H_2O_2 under physiological conditions. These H-donors could conceivably accelerate the decomposition of H_2O_2 by preventing the accumulation of the inactive $(Cat \cdot H_2O_2)_{II}$. The latter compound is not formed to a significant extent, however, in the system described by Chance (1952a).

Nitrite and alcohols enhance slightly the effectiveness of H_2O_2 as an inducer of catalase synthesis in Rps. spheroides (R. K. Clayton, unpublished). This can be expected from the foregoing considerations, since nitrite and alcohols should prolong the presence of the inducer.

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