

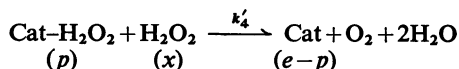
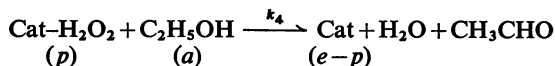
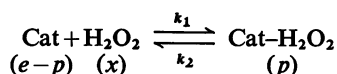
## APPENDIX

### Analysis of the Catalase-Hydrogen Peroxide Intermedi in Coupled Oxidations

By BRITTON CHANCE and NOZOMU OSHINO  
*Johnson Research Foundation, University of Pennsylvania,  
Philadelphia, Pa. 19104, U.S.A.*

(Received 13 September 1972)

In previous communications, we considered the kinetics of the catalase-H<sub>2</sub>O<sub>2</sub> intermediate in coupled oxidations of the isolated liver catalase (Chance, 1949) and of the mixed mitochondrial-peroxisomal fraction (Chance & Oshino, 1971). In those cases, it was possible to record the kinetics of formation of the intermediate by H<sub>2</sub>O<sub>2</sub> pulses, and solve for the H<sub>2</sub>O<sub>2</sub> concentration. In most of the cases, however, only the analysis of steady-state effects are feasible and will be useful for the further study on the catalase reaction, especially in a system such as the perfused liver. It seems appropriate, therefore, to extend the analysis. The chemical equations for the catalase reactions are:



Symbols\* in parentheses represent the concentrations of corresponding molecular species. In the following analysis the designations of rate constants and the symbols for molecular species do not conform with current practice but instead retain the nomenclature originally used, to avoid confusion and to simplify a comparison of these equations with those previously derived (Chance, 1949; Chance *et al.*, 1952).

The three differential equations for catalase action

\* The following symbols are used:  $p$ , concentration of the catalase-H<sub>2</sub>O<sub>2</sub> intermediate (Compound I) at any time, based on haem molarity;  $p_m$ , concentration of catalase-H<sub>2</sub>O<sub>2</sub> at the steady state;  $p_M$ , maximal attainable value of  $p_m$  for a particular  $e$  value;  $e$ , total concentration of enzyme, catalase haem iron molarity;  $a$ , concentration of hydrogen donor;  $x$ , concentration of H<sub>2</sub>O<sub>2</sub> at any time;  $x_m$ , concentration of H<sub>2</sub>O<sub>2</sub> at the steady state;  $\frac{dx_n}{dt}$ , rate of H<sub>2</sub>O<sub>2</sub> generation;  $p/e$ , proportion of haem binding with H<sub>2</sub>O<sub>2</sub> in the total haem.

(Chance *et al.*, 1952) are modified by inserting into eqn. (2) the rate of generation of H<sub>2</sub>O<sub>2</sub>,  $dx_n/dt$ :

$$\frac{dp}{dt} = k_1 x(e-p) - k_4' xp - (k_2 + k_4 a)p \quad (1)$$

$$\frac{dx}{dt} = \frac{dx_n}{dt} - k_1 x(e-p) - k_4' xp + k_2 p \quad (2)$$

$$\frac{da}{dt} = -k_4 ap \quad (3)$$

The equations are solved for the steady state of the catalase intermediate ( $\frac{dp}{dt} = 0$ ), for the steady state of H<sub>2</sub>O<sub>2</sub> generation ( $\frac{dx}{dt} = 0$ ) and for the particular case where  $k_2$ , the rate of dissociation of H<sub>2</sub>O<sub>2</sub> from catalase intermediate, is negligible. The initial concentration of the hydrogen donor is  $a_0$ . The equations were also studied by Clayton (1959) and a graphic solution representing the various parameters was presented. Summing eqns. (1) and (2), and noting that when  $\frac{dx}{dt} = 0$ ,  $x = x_m$  and  $p = p_m$ , the steady-state concentration of the intermediate (as distinguished from  $p_M$ , the maximum concentration of the intermediate), gives eqn. (4a):

$$\frac{dx_n}{dt} = 2k_4' x_m p_m + k_4 a_0 p_m \quad (4a)$$

The steady-state solution for  $p_m/e$  is derived from a solution of eqn. (1), giving eqn. (5a):

$$\frac{p_m}{e} = \frac{1}{1 + \frac{k_4'}{k_1} + \frac{k_4 a_0}{k_1 x_m}} \quad (5a)$$

In general terms,  $k_4' = nk_1$ , and eqn. (4a) becomes:

$$\frac{dx_n}{dt} = 2nk_1 x_m p_m + k_4 a_0 p_m \quad (4b)$$

Similarly, eqn. (5a) becomes:

$$\frac{p_m}{e} = \frac{1}{1+n+\frac{k_4 a_0}{k_1 x_m}} \quad (5b)$$

A general equation for  $\frac{dx_n}{dt}$  can be obtained by substituting eqn. (5b) into eqn. (4b) and eliminating  $x_m$ . From eqn. (5b):

$$x_m = \frac{k_4 a_0}{k_1} \left[ \frac{p_m}{e - (n+1)p_m} \right] \quad (5c)$$

then, from eqn. (4b) by substituting for  $x_m$  and rearranging:

$$\frac{dx_n}{dt} = k_4 a_0 p_m \left[ 1 + 2n \left( \frac{1}{\frac{e}{p_m} - (n+1)} \right) \right] \quad (4c)$$

This equation generally relates the rate of  $H_2O_2$  generation to the rate of the 'peroxidatic' reaction.

When, from eqn. (3),  $-\frac{da}{dt}$  is substituted for  $k_4 a_0 p_m$ :

$$\frac{dx_n}{dt} = \left( -\frac{da}{dt} \right) \left[ 1 + 2n \left( \frac{1}{\frac{e}{p_m} - (n+1)} \right) \right] \quad (4d)$$

Rearranging:

$$\frac{p_m}{e} = \frac{1}{n+1+2n \left[ \frac{\frac{dx_n}{dt}}{\left( -\frac{da}{dt} \right)} - 1 \right]} \quad (4e)$$

Therefore the  $p_m/e$  value directly relates to the proportion of the 'peroxidatic' reaction in the overall  $H_2O_2$ -decomposition reaction. Experimentally a linear relationship is obtained and the deviation from theory (eqn. 4e) is largely set by the error of the experiments from  $p_m/e = 0.02$  to 0.39 as shown in Fig. 1.

Simplified forms of eqn. (4c) are obtained if  $a_0$  is adjusted to  $a_{1/2}$ , the value that brings  $p_m$  to  $p_m/2$ . From eqn. (5b) as  $x_m \rightarrow \infty$ ,  $p_m \rightarrow p_M$ , thus:

$$\frac{p_M}{e} = \frac{1}{1+n} \quad (5d)$$

At  $a_0 = a_{1/2}$ ,

$$p_m = \frac{p_M}{2} = \frac{e}{2(1+n)} \quad (5e)$$

Substituting for  $p_m$  in eqn. (4c) and simplifying:

$$\left[ \frac{dx_n}{dt} \right]_{a_{1/2}} = k_4 a_{1/2} p_m \left( \frac{3n+1}{n+1} \right) = k_4 a_{1/2} e \left[ \frac{3n+1}{2(n+1)^2} \right] \quad (4f)$$

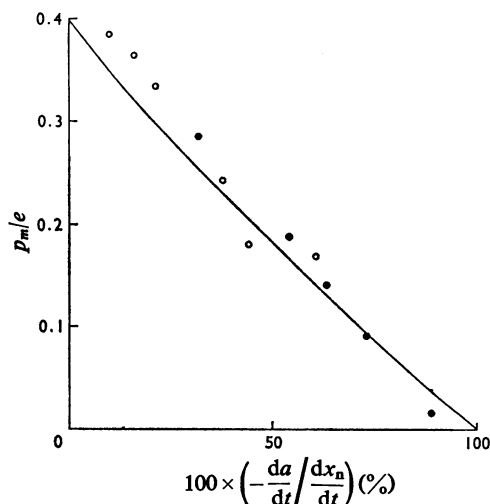


Fig 1. Relationship between  $p_m/e$  and  $\frac{da}{dt} / \frac{dx_n}{dt}$

The steady-state concentration of the catalase- $H_2O_2$  intermediate ( $p_m/e$ ) and the proportion of the 'peroxidatic' reaction ( $da/dt$ ) in the overall  $H_2O_2$  decomposition ( $dx_n/dt$ ) were measured as described for Fig. 7 and Fig. 1 in the main paper (Oshino *et al.*, 1973b) respectively. —, Theoretical curve of eqn. (4e). o, Experimental values in the presence of 1 mM-ethanol; ●, experimental values in the presence of 10 mM-ethanol.

Eqn. (4f) is rearranged:

$$a_{1/2} = \left[ \frac{1}{3n+1} k_4 \right] \frac{1}{e} \frac{dx_n}{dt} \quad (4g)$$

For rat liver catalase  $n = 1.5$  (Chance & Oshino, 1971) and, assuming  $k_4$  for ethanol =  $10^3 M^{-1} \cdot s^{-1}$  (Chance, 1949), eqn. (4g) becomes:

$$a_{1/2} = 2.28 \times 10^{-3} \left( \frac{1}{e} \frac{dx_n}{dt} \right) \quad (4h)$$

Thus the  $a_{1/2}$  value can represent the ratio of  $H_2O_2$ -generation rate to the catalase concentration in the system. An experimental support for eqn. (4f) was obtained;  $k_4$  values for ethanol and methanol calculated from eqn. (4f) by measuring the  $\frac{1}{e} \frac{dx_n}{dt}$  and  $a_{1/2}$  values are in good agreement with directly determined values (Chance, 1949) (Table 1).

In a similar treatment, eqns. (4d) and (4f) become:

$$\frac{dx_n}{dt} = \left( -\frac{da}{dt} \right) \left( 1 + \frac{3}{\frac{e}{p_m} - 2.5} \right) \quad (4i)$$

Table 1.  $k_4$  values for methanol and ethanol estimated from eqn. (4f)

The alcohol concentration producing  $p_m/e = 0.2$  at various  $\frac{1}{e} \frac{dx_n}{dt}$  values was determined as described for Fig. 7 in the main paper (Oshino *et al.*, 1973b). Since  $n = 1.5$  with rat liver catalase,  $k_4$  was calculated from the equation:  $\frac{1}{e} \frac{dx_n}{dt} = 0.44 k_4 a_{1/2}$ . The temperatures used for methanol and ethanol experiments were 30°C and 25°C respectively. The  $k_4$  values for methanol and ethanol, which were directly measured at room temperature, were  $10^3 \text{ M}^{-1} \cdot \text{s}^{-1}$  (Chance, 1949).

$\frac{1}{e} \frac{dx_n}{dt}$ (s <sup>-1</sup> )	Alcohol	$10^3 \times a_{1/2}$ (M)	$10^{-3} \times k_4$ (M <sup>-1</sup> ·s <sup>-1</sup> )
0.067	Methanol	0.10	1.5
0.096		0.20	1.1
0.125		0.30	1.0
0.200		0.40	1.1
0.322		0.60	1.2
0.200	Ethanol	0.5	0.9
0.367		1.0	0.8
1.02		3.0	0.8
2.40		6.0	0.9
4.84		10.0	1.1

$$\left[ \frac{dx_n}{dt} \right]_{a_{1/2}} = -2.2 \left[ \frac{da}{dt} \right]_{a_{1/2}} \quad (4j)$$

Eqns. (4i) and (4j) define the relationship between the rate of H<sub>2</sub>O<sub>2</sub> generation and the rate of alcohol disappearance or aldehyde formation under conditions where the hydrogen donor,  $a_0$ , has been adjusted to a value of  $a_{1/2}$ : where the steady-state concentration of the catalase intermediate,  $p_m$ , is one-half its maximum concentration, i.e.  $p_m = p_M/2$ , and assuming that a steady state can be maintained, i.e.  $dx/dt = 0$ . The latter represents a significant constraint upon the system, and implies that there is enough catalase present to 'control' the H<sub>2</sub>O<sub>2</sub> concentration and maintain it at a constant value. When this constraint is applicable, then it is apparent that the fraction of the H<sub>2</sub>O<sub>2</sub> generated that is utilized in converting alcohol into aldehyde in the presence of sufficient donor to give  $p_m = p_M/2$  is 45% of the total, for a catalase for which  $m = 1.5$  (eqn. 4j) [cf. Fig. 3 in the main paper (Oshino *et al.*, 1973b)]. The data further show that the fractional conversion is independent of the rate of H<sub>2</sub>O<sub>2</sub> generation and of the molarity of the enzyme (Sies *et al.*, 1973).

The steady-state H<sub>2</sub>O<sub>2</sub> concentration,  $x_m$ , can further be related to the rate of alcohol disappearance or

aldehyde formation by the following series of steps. Eqns. (5c) and (5e) are arranged:

$$(n+1)k_1 x_m = k_4 a_{1/2} \quad (6)$$

From eqns. (3) and (5e),

$$k_4 a_{1/2} = -\frac{2(n+1)}{e} \frac{da}{dt} \quad (7)$$

Substituting eqn. (7) into eqn. (6):

$$(n+1)k_1 x_m = -\frac{2(n+1)}{e} \frac{da}{dt} \quad (8a)$$

Simplifying:

$$\frac{da}{dt} = \left[ -\frac{k_1 x_m e}{2} \right]_{a_{1/2}} \quad (8b)$$

In this equation, it is observed that the rate of alcohol disappearance or aldehyde formation at a hydrogen donor concentration that causes  $p_m = p_M/2$  is, first, independent of the value of  $n$  for the particular catalase. Secondly, it is constant for a wide range of  $x_m$  values and  $e$  values. In this respect, the system is self-adjusting; with low values of  $e$ ,  $x_m$  will be high and vice versa. Thus we find an explanation for the insensitivity of alcohol disappearance or aldehyde formation to the washing of catalase out of microsomal fraction (Roach *et al.*, 1969) or to the inhibition of catalase by cyanide or azide; only the balance between  $x_m$  and  $e$  values is altered.

By substituting  $da/dt$  in eqn. (4f), with its  $k_4 a_{1/2} p_m$  value in eqn. (8b), we get:

$$\left[ \frac{dx_n}{dt} \right]_{a_{1/2}} = \left( \frac{3n+1}{n+1} \right) \left( \frac{k_1 x_m e}{2} \right) \quad (9a)$$

or:

$$\left[ \frac{1}{e} \frac{dx_n}{dt} \right]_{a_{1/2}} = \left( \frac{3n+1}{n+1} \right) \left( \frac{k_1 x_m}{2} \right) \quad (9b)$$

Thus the term  $\frac{1}{e} \frac{dx_n}{dt}$  is linearly related to  $x_m$  when  $a_0 = a_{1/2}$ .

The agreement of the equations developed here with the experimental results on coupled oxidations gives further support to the validity of the general equations for the 'catalatic' and 'peroxidatic' reactions of catalase (Chance *et al.*, 1952; Chance, 1969), and provides the theoretical basis for applying the steady-state analysis in the perfused liver (Sies *et al.*, 1973; Oshino *et al.*, 1973a).

This work was supported by Research Grant MH-20573-1 from the National Institute of Mental Health, U.S.A.

#### References

- Chance, B. (1949) *Acta Chem. Scand.* **1**, 236-267  
 Chance, B. (1969) *Ann. N.Y. Acad. Sci.* **168**, 354-355  
 Chance, B. & Oshino, N. (1971) *Biochem. J.* **122**, 225-233

- Chance, B., Greenstein, D. S. & Roughton, F. J. W. (1952)  
*Arch. Biochem. Biophys.* **37**, 301-339
- Clayton, R. K. (1959) *Biochem. Biophys. Res. Commun.* **1**,  
191-193
- Oshino, N., Chance, B., Sies, H. & Bücher, Th. (1973a)  
*Arch. Biochem. Biophys.* **154**, 117-131
- Oshino, N., Oshino, R. & Chance, B. (1973b) *Biochem. J.*  
**131**, 555-563
- Roach, M. K., Reed, W. N. & Creaven, P. J. (1969) *Biochem. Biophys. Res. Commun.* **36**, 569-602
- Sies, H., Bücher, Th., Oshino, N. & Chance, B. (1973)  
*Arch. Biochem. Biophys.* **154**, 106-116