The Catalatic Decomposition of Hydrogen Peroxide and Its Retardation by Azide

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It is confirmed that at low concentrations of hydrogen peroxide its catalatic decomposition follows a first order law. A linear relationship between the reciprocal rate constant and the concentration of azide ion is found. From the experiments the equilibrium constant for the equilibrium between an inactive catalase-azide complex and active catalase is determined.

Zeile and Hellström¹ have shown that catalase is a hemoprotein in which the hematin acts as a prosthetic group.

In the present work horse kidney catalase is used. According to Bonnichsen² it has four heme groups per molecule, while horse liver catalase contains three hemines and one verdohemochromogen group.

Several theories have been advanced to explain the decomposition of hydrogen peroxide by catalase. Theorell³ and Chance⁴ both find that two hydrogen peroxide molecules react with one catalase molecule. According to Theorell each hydrogen peroxide molecule is attached to one hematin group and from two such groups oxygen and water are subsequently released. Chance, however, assumes that one hydrogen peroxide molecule is bound to one hematin group and that this group then reacts with a free hydrogen peroxide molecule.

The decomposition of hydrogen peroxide by means of catalase is a first-order reaction provided the catalase concentration is about $10^{-9} M$. Svendsen⁷ has found that a first-order reaction is also obtained if the catalase concentration is about $10^{-10} M$ provided the substrate concentration is low.

Experiments at low hydrogen peroxide concentrations (*ca.* $10^{-3} M$) were carried out and confirmed that the enzyme under these circumstances is not (or at least only very slowly) inactivated by the hydrogen peroxide: The first-order reaction constant was determined from 4—5 titrations during a time corresponding to one or two half-periods. After a further 5—6 half-periods fresh hydrogen peroxide was added to make the concentration up to about the original and measurements were again taken over about two half-periods to give a new determination of the rate constant. Most frequently the two

determinations were identical; still it happened that the last was only say eighty per cent of the first. But according to Svendsen⁸ this decrease in activity is probably not an irreversible inactivation, but due to adsorption of the catalase to the glass-walls, setting in slowly when the hydrogen peroxide is nearly used up. At least within two half-periods the catalase is of practically constant activity and the first-order constant measures its concentration.

Table 1.

(Exp. 15 24/10, 52. $t = 20^\circ \text{C}$. $C_{\text{Catalase}} = 10^{-10} \text{ M}$. $\text{CH}_2\text{O}_2 \sim 10^{-3} \text{ M}$. $\text{pH} = 7.0$)
($\text{CNa}_2\text{HPO}_4 = 1/1500 \text{ M}$; $\text{CKH}_2\text{PO}_4 = 1/1500 \text{ M}$.)

t_{min}	ml Cerisol/g sol. = $p \text{ CH}_2\text{O}_2$	$\log p \text{ C}$	$\frac{\log C_0}{C}$	$\frac{1}{0.0625} \log \frac{C_0}{C} (= t)$
0	0.0518	0.7143-2	0	
1	0.0448	0.6513-2	0.0630	1.01
2	0.0388	0.5888-2	0.1255	2.01
4	0.0293	0.4669-2	0.2474	3.96
7	0.0191	0.2810-2	0.4333	6.95
	new H_2O_2 added at $t = 29$			
0	0.0592	0.7723-2	0	
1	0.0509	0.7076-2	0.0647	1.04
3	0.0387	0.5877-2	0.1846	2.96
5	0.0292	0.4654-2	0.3069	4.91
8	0.0186	0.2695-2	0.5028	8.05

$$k' = 0.0625$$

The catalase was prepared from horse kidney after Bonnichsens prescription⁵. It was kept in a $ca. 2 \cdot 10^{-8} \text{ M}$ solution with 0.01 M potassium chloride added to prevent adsorption to the glass walls (Svendsen⁸); the activity is then constant for some months. The catalase molarity was determined with a Beckman-Spectrophotometer, setting the extinction in millimolar solution at $405 \text{ m}\mu$ and 1 cm column to 380.

In Table 1 $k' = 0.0625$ is the constant at 20°C using decadic logarithms and the minute as time-unit and with a catalase molarity of 10^{-10} . Therefore:

$$k = \frac{0.0625}{0.4343} \cdot \frac{1}{60} \cdot 10^{10} = 2.40 \cdot 10^7 \text{ l} \cdot \text{moles}^{-1} \cdot \text{sec}^{-1}$$

This value is smaller than Svendsen's value: $2.65 \cdot 10^7$ (also for horse kidney catalase) and much less than that of Bonnichsen: $3.5 \cdot 10^7$ (for horse erythrocyte catalase). This is probably due to impurities in the catalase preparation, which was not perfectly crystalline.

The influence of azide on the first-order rate constant k' was studied in the following way, using Svendsen's⁷ technique. Buffer-solution ($1/1500 \text{ M}$ $\text{Na}_2\text{HPO}_4 + 1/1500 \text{ M}$ KH_2PO_4 , $\text{pH} \sim 6.95$), peroxide and azide are mixed in a black-painted reaction flask; at the time t_0 catalase is added from a micro-pipette, the flask is shaken, put into the thermostat at 20°C and the stop-watch is started. At intervals a sample is withdrawn, blown into a weighed flask containing enough 5 M sulphuric acid to make the solution about 0.5 M and weighed. Hydrogen peroxide is then titrated with 0.02 M ceric sulphate using "Ferroin" as indicator.

The konstant k' was determined with azide-concentrations varying from 10^{-8} to $1.2 \cdot 10^{-6} M$ and with $\text{pH} \sim 6.95$. Plotting C_{azide} against $1/k'$ one gets a straight line which indicates the soundness in assuming a mass-action law behavior. Setting

$$\frac{C_{\text{active catalase}} \cdot C_{\text{azide}}}{C_{\text{inactive catalase}}} = K$$

and

$$\frac{C_{\text{active catalase}}}{C_{\text{inactive catalase}}} = \frac{k'}{k_0' - k'}$$

we get

$$C_{\text{azide}} = \frac{Kk_0'}{k'} - K$$

$k_0' = 0.0645$ was found by extrapolation to $C_{\text{azide}} = 0$, consistent with the value found in Table 1 (0.0625) and other experiments without azide. $K = 0.92 \cdot 10^{-7} M$ was found from the slope of the line ($= K \cdot k_0'$).

Table 2. Comparison between the experimental values of k' for different azide concentrations and values calculated from

$$k'_{\text{calc.}} = \frac{0.92 \cdot 10^{-7} \cdot 0.0645}{C_{\text{azide}} + 0.92 \cdot 10^{-7}}$$

($\text{pH} = 6.95 \pm 0.05$; $T = 20^\circ \text{C}$).

Azide conc.'s in moles/liter	k'_{exp}	$k'_{\text{calc.}}$	% deviation
12.05 10^{-7}	0.00448	0.00457	+ 2.0
10 —	0.00521	0.00543	+ 4.1
9 —	0.00604	0.00598	— 1.0
8 —	0.00641	0.00665	+ 3.6
6 —	0.00843	0.00857	+ 1.6
5 —	0.0103	0.01001	— 3.0
4 —	0.0131	0.01206	— 8.6
2 —	0.0211	0.0203	— 4.0
1 —	0.0299	0.0309	+ 3.2
0.5 —	0.0405	0.0418	+ 3.1
0.1 —	0.0614	0.0582	— 5.5
0	(0.0645) extrapol.		

Table 2 shows that a simple equilibrium is established between catalase and azide (at azide concentrations below $1.2 \cdot 10^{-6} M$) with a mass action constant $K = 0.92 \cdot 10^{-7}$ moles/l at $\text{pH} = 7$ *. The interesting point is that one

* From a recent paper of Chance * one extrapolates the value $K \sim 10^{-8}$ ($\text{pH} = 7$), apparently for the same constant. The great difference between the two values seems to indicate, that our K is the mass-action constant of an equilibrium between azide plus a complex of catalase with hydrogen peroxide on one side and a complex with both hydrogen peroxide and azide on the other, whereas no hydrogen peroxide enters the equilibrium measured by Chance.

Our constant depends also on pH ; the correct form for the equilibrium may therefore be:
active hydrogen peroxide catalase + $\text{N}_3^- \rightleftharpoons$ inactive complex + OH^-

The COH^- (at constant $\text{pH} = 7$) is incorporated in K .

single azide-ion is capable of inactivating the whole catalase molecule with its four hemin groups; these four groups must therefore be considered as a unity in spite of the fact, that the catalatic activity against hydrogen peroxide is not depending upon the existence of four groups; in horse-liver catalase there are only three and in spinach catalase one or two ¹⁰.

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