ACTA CHEMICA SCANDINAVICA 7 (1953) 545–550

Decomposition of Hydrogen Peroxide by Catalase

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Fifty years ago Senter [1] showed that the decomposition of hydrogen peroxide by catalase from erytrocytes followed a first order reaction, in case the substrate concentration was sufficiently low. At higher substrate concentration the reaction velocity constant decreased during the reaction. In the following years these results were not supported, to the contrary it was demonstrated that catalase was a very fragile enzyme and that it was inactivated by hydrogen peroxide during the reaction.

A few years ago Bonnichsen, Chance and Theorell [2] demonstrated that the reaction did follow the expression for a first order reaction. They used catalase in concentration about fifty times higher than previously and the activity was measured by rapid manipulation of the titration technique or a special polarographic method.

This paper supports the results of Senter and in addition it is shown that under special experimental conditions it is possible to repeat the reaction by further addition of hydrogen peroxide without change in the first order reaction velocity constant.

METHOD

Catalase was prepared from horse kidney after the method of Bonnichsen [3]. Perhydrol "Merck" diluted with redistilled water was used as substrate. Buffer solution $M/150$ phosphate "Sørensen", pH 7.0, temperature 20.0° C. The reaction flask (Pyrex) was treated with chromic-sulfuric acid cleaning mixture and thoroughly washed with redistilled water. After this the flask was inverted over a glass tube through which steam was flowing, so that the inside was rinsed by the condensed water. Between the experiments the flask was only washed with glassdistilled water and treated with steam. The diluted solution of hydrogen peroxide was stable in flasks treated in this way, the spontaneous decomposition being less than 0.5 per cent in twentyfour hours.

The reaction was started by adding the measured amount of catalase solution in a "watchglass" made from the bottom of ordinary test tubes. The reaction was followed by titration of samples at different times. Due to the content of oxygen bubbles in the

reacting mixture it was impossible to take out an accurate volume of the solution. According to Sten Andersen [4] the sample was taken up in a 20 ml pipette with a fairly large delivery orifice. The sample was at once allowed to run into a previously tared Erlenmeyer flask, which contained sulfuric acid in such an amount that the final concentration was 0.5 *M*. The flask was weighed within an accuracy of 0.01 g.

The hydrogen peroxide was titrated with potassium permanganate or ceric sulfate in both cases using Ferroin as indicator. The solution of Ferroin used was 1/400 *M*, which showed to be stable for some weeks. 25 μl of this solution was found to be a sufficient amount to give a distinct endpoint, at the same time requiring only a small correction for the titration of the indicator.

In the titration of the most diluted solutions of hydrogen peroxide with potassium permanganate it was necessary to add manganous salt just before the titration. A total concentration about 10^{-4} *M* of manganous sulfate was used.

EXPERIMENTAL

In some experiments with a catalase concentration of about 2×10^{-10} *M* it was found that high concentrations of hydrogen peroxide inactivated the enzyme. That was not the case at low concentrations of hydrogen peroxide.

The catalase was added to the buffered hydrogen peroxide solution, which had been equilibrated in a water bath at 20.0° C. After six half changes or more the hydrogen

Table 1. The variation of the velocity constant in successive experiments. The reaction at time 0 has been started by adding catalase, the other by adding hydrogen peroxide at the initial concentration designed a. For explanation see text.

Time in hours	k'	a
0	0.311	0.0024 *M*
1.5	0.233	0.0023 »
25	0.198	0.0020 »
25.5	0.198	0.0013 »
0	0.309	0.0024 »
0.5	0.284	0.0024 »
50	0.136	0.0024 »
51	0.136	0.0025 »
0	0.325	0.0015 »
0.5	0.307	0.0015 »
24	0.265	0.0014 »
24.5	0.263	0.0014 »
48	0.216	0.0015 »
48.75	0.215	0.0015 »
49.5	0.215	0.0015 »

Table 2. The velocity constant with varying concentration of catalase.

Initial hydrogen peroxide concentration	Catalase concentration	k
0.0019 M	$3.33 \times 10^{-10} M$	2.64×10^7 l, mole^{-1}sec^{-1}
0.0019 »	$1.67 \times 10^{-10} M$	2.68×10^7
0.0019 »	$8.36 \times 10^{-11} M$	2.63×10^7 *
0.0019 »	$4.20 \times 10^{-11} M$	2.60×10^7 *
0.0005 »	$3.33 \times 10^{-10} M$	2.85×10^7
0.0005 »	$1.67 \times 10^{-10} M$	2.73×10^7
0.0005 »	$8.36 \times 10^{-11} M$	2.53×10^7
0.0005 »	$4.20 \times 10^{-11} M$	2.60×10^7
	Average	2.65×10^7 l, mole^{-1}sec^{-1}

* The velocity constant has been calculated from the first half change.

peroxide was brought to the initial concentration by adding hydrogen peroxide in such a concentration that the dilution effect on the catalase concentration could be ignored.

With an initial hydrogen peroxide concentration of 0.1 M the catalase could decompose only fifty per cent, whereas it decomposed all the hydrogen peroxide when the initial concentration was 0.05 M. When the hydrogen peroxide again was brought to 0.05 M no reaction occurred.

By gradually decreasing the initial substrate concentration the catalase subsisted through more runs before total inactivation occurred. With an initial substrate concentration of 0.01 M the inactivation was complete after the fourth addition of hydrogen peroxide. In experiments with an initial substrate concentration of 0.005 M the activity decreased only about 35 per cent during four runs. In experiments with an initial substrate concentration like this the reaction followed a first order reaction during the first or the first and second half change. With initial substrate concentration of 0.0024 M or lower the reaction followed a first order reaction in its whole course.

By repeating the reaction it was found that the velocity constant after a certain decrease did not change during two or three runs. The results are given in Table 1.

In some experiments the catalase concentrations were determined photometrically just before the measurement of activity by taking the extinction as ε (405 mμ, 10^{-3} M) = 380 (Bonnichsen [5]).

Using 0.0019 M hydrogen peroxide the reaction was first order when the catalase concentration was 1.67 and 3.33×10^{-10} M. With a catalase concentration of 4.2 and 8.3×10^{-11} M the velocity constant decreased after the elapse of the first half change. Calculation of the velocity constant during the first half change gave the same value as that found in experiments with initial substrate concentration of 0.0005 M, in which the reaction followed the expression for a first order reaction from six to more than 95 per cent decomposition of hydrogen peroxide (Fig. 1).

DISCUSSION

Using catalase prepared from horse kidney it is shown that the decomposition of hydrogen peroxide follows a first order reaction at a catalase con-

centration from 4.2×10^{-11} M to 3.33×10^{-10} M if the hydrogen peroxide concentration is sufficiently low. The results confirm Senter's experiments.

The reaction is described by the expression for a first order reaction

$$\ln \frac{a}{x} = k \cdot E \cdot t$$

where t is in seconds, E is the molar enzyme concentration, a is the initial substrate concentration at $t = 0$, and x is the substrate concentration at the time t.

In some experiments where the enzyme concentration was not known with sufficient accuracy the following expression has been used

$$\ln \frac{a}{x} = k' \; t$$

where t is in minutes and

$$k' = 60 \; E \cdot k$$

Table 2 gives the values of the velocity constants which will be seen to agree fairly well. The lower values than that previously found by Bonnichsen [5], 3.5×10^7 1 $mole^{-1}sec^{-1}$, are perhaps due to the fact that the catalase solution at the time of measuring had been kept in refrigerator for several months.

The inactivation of catalase by the substrate is very well known from the literature. It is possible to get rid of it in two ways, either by increasing the enzyme concentration as used by Bonnichsen *et al.*[2] and Beers and Sizer [6] or by decreasing the substrate concentration. It is possible that a catalase concentration lower than 4×10^{-11} M at 20° will require a substrate concentration lower than 0.0005 M.

Many years ago it was assumed that the oxygen set free during the reaction was the agent which caused the inhibition. In experiments at atmospheric pressure and with hydrogen peroxide concentration of about 0.05 M the formation of oxygen is so fast that the solution is saturated with oxygen to such an extent that it is equivalent to a solution saturated with oxygen under pressure of several atmospheres. This is the case before bubbles of oxygen can be seen. Decreasing the total pressure over the solution to about 80 mm of mercury and thorough aeration does not change the course of the reaction. Therefore one must conclude in agreement with Michaelis and Pechstein [7] that the inactivation is not due to oxygen.

Previously it has been observed that catalase solutions, especially if dilute, will show a decreasing activity upon standing. As the decrease is more pronounced when the glass surface is increased by adding glass beads (Bonnichsen *et al.*) it has been ascribed to an adsorption phenomenon.

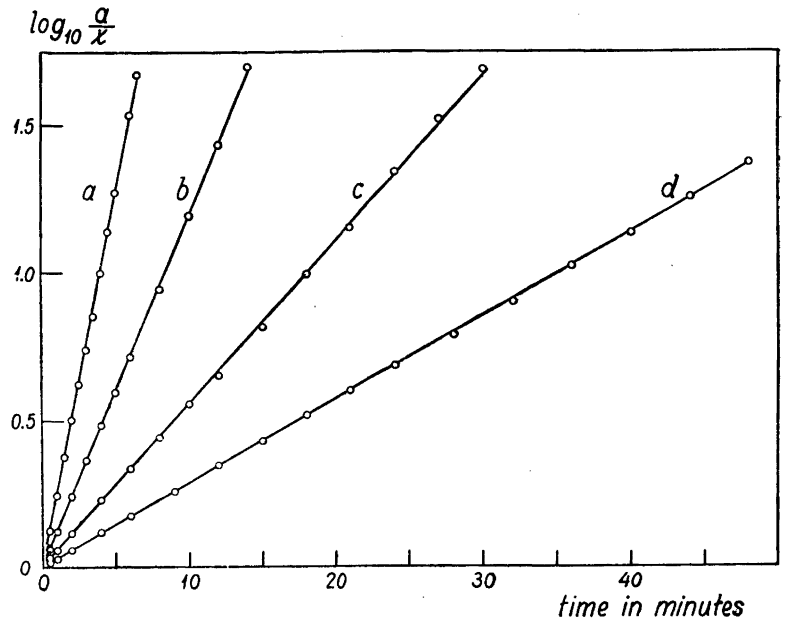

Fig. 1. First order rate curves of decomposition of hydrogen peroxide by varying concentrations of catalase ($a = 3.33 \times 10^{-10}$, $b = 1.67 \times 10^{-10}$, $c = 8.36 \times 10^{-11}$ and $d = 4.20 \times 10^{-11}$ M). Initial concentration of hydrogen peroxide 0.0005 M. Temperature 20.0° C, pH 7.0, M/150 phosphate buffer.

The decrease of k' in Table 1 is probably caused by adsorption of catalase at the glass surface. If the decrease in catalase concentration caused by adsorption is small during the time it will take to run two or three kinetic experiments, one would expect an approximately equal velocity constant in the experiments. Table 1 shows that in 24 hours the adsorption will proceed at a sufficiently low rate to make k' a real constant.

These experiments thus only demonstrate in another way that at low hydrogen peroxide concentration the catalase is not inactivated by the substrate.

As shown the reaction follows the expression for a first order reaction from six to more than 95 per cent decomposition of the substrate, which indicates that the adsorption phenomenon does not take place in solutions in which there is a measurable amount of hydrogen peroxide. As the activity is decreased in the reaction flask from the first run to the second and third, one must assume that it is the hemin group, which is adsorbed on the glass surface.

SUMMARY

Using catalase prepared from horse kidney it is shown that inactivation by the substrate does not take place in case the initial hydrogen peroxide concentration is sufficiently low, and under these circumstances the reaction is a first order reaction.

The support of a grant from "*Købmand i Odense Johann og Hanne Weimann, f. Seedorffs legat*" was of great help.

The author wishes to thank professor J. A. Christiansen, Institute of Physical Chemistry, University of Copenhagen, for introduction into the problems and for his constant interest and advice.

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Received January 29, 1953.