Adsorption in Solutions of Catalase

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It is known that dilute solutions of catalase upon standing will show a decrease in activity. The decrease is accentuated when the glass area is increased by addition of glass beads (K. Agner, personal communication to Bonnichsen *et al.*¹). Previously it was shown by Harkins, Fourt and Fourt² that catalase is adsorbed in a monolayer at various interfaces. It has not been possible to find any information on how to minimise the adsorption in dilute solutions.

In kinetic experiments it is of interest to minimise the adsorption or at least to have some information about it, and some experiences will be reported.

METHOD

As previously described the decomposition of hydrogen peroxide by catalase follows a first order reaction in case the substrate concentration is low³. As a measure of activity or concentration of catalase the velocity constant is used as taken from the expression

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

where a is the substrate concentration at $t = 0$, x the substrate concentration at the time t (in minutes).

The substrate, Perhydrol "Merck", was used at a concentration of 0.0019 M or lower. The activity measurement was carried out at 20.0° C and at pH = 7.0 ($M/150$ phosphate "Sørensen"). The measurement of k' was usually based upon four to eight samples in each activity determination. From the catalase solution to be tested, an amount was taken for activity determination, such that k' was about 0.2 to 0.3. If it was lower more samples were taken.

The catalase was prepared from horse kidney according to Bonnichsen⁴. A solution with a molar concentration of catalase about 4×10^{-6} was diluted to give the desired concentration. The activity determination was started within one minute from the dilution. The amount of catalase used for activity determination was measured with a pipette,

which was blown out into the reacting mixture. In several experiments the initial k' only varied about two per cent. Some experiments, in which the catalase was measured by means of a Krogh syringe, the initial k' showed a great variation, probably caused by adsorption at the ground glass surface of the syringe.

The area of glass surface, which is in contact with the catalase solution, is taken from the internal diameter of the flask and the height of the solution.

EXPERIMENTAL

In the first experiment an attempt was made to elute the adsorbed catalase. After standing for two weeks a solution of catalase in distilled water showed $k' = 0.295$. Potassium chloride was added and chloride titration showed 24 meq./l of chloride *. Two hours later $k' = 0.404$ showing that the activity was increased about 37 per cent.

A 25 ml volumetric flask, in which a catalase solution had been stored for seven days, was washed once with distilled water, and filled to the mark. Fifteen minutes later the content of the flask was poured into a hydrogen peroxide solution for activity determination, which gave a k' of 0.016. The flask was then filled with 0.01 *M* KCl, and fifteen minutes later the activity was determined as before. This time the $k' = 0.081$ or about five times as high as when washing had been done with pure water.

Table 1. Activity determination in solutions of catalase with molar concentrations at 6.9×10^{-8} .

Time	Catalase dissolved in	
	H ₂ O k'	0.01 <i>M</i> KCl k'
0	0.301	0.303
1 hour	0.297	0.303
1 day	0.276	0.286
2 days	0.263	
3 »	0.250	0.285
4 »	0.251	0.290
5 »	0.248	0.294
7 »	0.246	0.290
8 »	0.255	0.286
9 »	0.245	
10 »		0.292
12 »	0.247	0.289

After addition of potassium chloride (chloride concentration 1.3 meq./l).

15 minutes	0.296
1 hour	0.307
1 day	0.284
57 days	0.275

* The chloride determinations have kindly been performed by Dr. Barker Jørgensen and Mr. P. Rosenkilde.

Table 2. Activity determination in solutions of catalase with molar concentration at 6.9×10^{-9} .

Time	Catalase dissolved in H_2O	
	without stirring	with stirring
0	0.303	0.312
65 minutes	0.303	
85 »		0.288
150 »	0.307	
1 day	0.291	0.248
3 days		0.231
4 »	0.249	
5 »		0.218
6 »	0.221	0.216
7 »	0.218	
8 »		0.205
10 »	0.198	
14 »	0.173	
35 »	0.127	

After addition of potassium chloride to 0.01 *M*.

1 hour	0.227
1 day	0.233

Solutions stored in flasks made from ordinary glass sometimes will show a small increase in activity some days after the initial decrease. Because of this it was decided to use only new flasks made from "Pyrex" glass. The flasks were treated as previously described ³.

In the flasks in which the catalase was diluted to a molar concentration at 6.9×10^{-8} with distilled water, the activity was decreased to 82 per cent of the initial. Equilibrium was reached in four days and afterwards no changes occurred for nearly two weeks. By that time a few milligrams of potassium chloride or sodium chloride were added. The final chloride concentration was determined by titration. It varied from 0.6 to 1.3 meq./l. Samples withdrawn within fifteen minutes to two hours after the addition of salt showed that the activity had increased again to between 96 to 102 per cent of the original. During the following twentyfour hours the activity decreased to about 95 per cent.

A similar decrease is found when catalase is dissolved in 0.01 *M* KCl or NaCl to 6.9×10^{-8} *M*. In such solutions equilibrium was reached in a day and remained unchanged for at least 12 days. In Table 1 the results from two experiments are given.

Dilution of the catalase with 0.005 *M* sodium or potassium sulfate, *M*/15 or *M*/150 phosphate (Sørensen) at pH = 7.0 seemed to inhibit the adsorption to a similar degree. Dilution with 0.01 *M* sodium acetate however gave an activity loss of ten per cent.

A solution of catalase diluted with water to 6.9×10^{-8} *M* and stored in a flask made from polyethylene showed a decrease in activity to about 75 per cent in thirteen days. Addition of potassium chloride to a concentration at 1.02 meq. increased the activity to 85 per cent of the initial value.

Fig. 1. The rate of reaction in a solution of catalase in which the decrease in activity was allowed to go far, before the reaction was started by adding hydrogen peroxide.

In solutions diluted to a molar concentration at 6.9×10^{-9} it was not possible to reach equilibrium (Table 2) during the period of observation. Difficulties in avoiding bacterial contamination restricted the extension of the experiments in time.

In a catalase solution of molar concentration 1.7×10^{-10} , the loss in activity was about twenty five per cent during the first hour, and forty per cent in the following twenty hours. Addition of potassium chloride (0.01 *M*) or phosphate buffer (pH = 7.0) at a total concentration at *M*/150 did not change these results. In these experiments the adsorption was allowed to take place in the reaction flask.

A catalase solution with a molar concentration at 1.6×10^{-10} was stored for three days at 20.0 °C. The initial activity was determined in an experiment carried out immediately after the dilution ($k' = 0.298$). After the elapse of three days hydrogen peroxide was added to make a concentration of 0.0018 *M*. The reaction was followed by taking several samples. The rate of the reaction is shown in Fig. 1. From the first, second and third sample the k' equalled 0.101, or 34 per cent of the initial. At that time about ten per cent of the hydrogen peroxide had been decomposed. The k' increased from this value to 0.177 and remained constant from about 60 to more than 95 per cent decomposition of the hydrogen peroxide.

In a similar experiment the k' value increased during the reaction from 28 to about 48 per cent of the initial.

DISCUSSION

The experiments show that the activity decreases in dilute solutions of catalase. The decrease depends to a high degree upon the initial concentration of catalase. As it is possible by elution to bring about a return of the activity,

in some cases to the initial level, one may conclude, that the decreased activity is mainly caused by adsorption and not by other types of inactivation.

Assuming the catalase molecules to be adsorbed in a monolayer² we can calculate the cross section of the molecule. Three experiments with a molar concentration of catalase of 6.9×10^{-8} showed a 17–18 per cent decrease, corresponding to adsorption of 17.7×10^{13} molecules on a glass surface of about 36 cm² indicating a cross section of the catalase molecule about 2 000 square Å. From another experiment with half the catalase concentration (3.4×10^{-8} M) the cross section was calculated to be 2 100 square Å.

A comparison of the adsorbed catalase molecule with molecules of simpler composition will give an impression of the result and its order of magnitude: Palmitic acid with a molecular weight (256) approximately 1/1 000 of the catalase (225 000), has a cross section about 21 square Å⁵. If the molecular weight of the palmitic acid were increased a thousand times the cross section would be increased a hundred times, or to 2 100 square Å, a value which agrees with that found for the catalase molecule.

At equilibrium one finds in saltfree solutions from the measurements that each cm² carries about 5×10^{12} molecules of catalase. Upon addition of salt or dilution of the catalase with salt solutions, the equilibrium is displaced, so that only 1.5×10^{12} molecules/cm² remains adsorbed.

In saltfree solutions where the molar concentration of catalase was 6.9×10^{-9} it was not possible to obtain equilibrium. A calculation shows that nearly all catalase molecules in solution could be adsorbed on the glass surface, carrying about 5×10^{12} molecules on each cm².

The addition of potassium chloride causes a change in the adsorption, and if one is allowed to assume equilibrium this is reached when each cm² carries 1.3×10^{12} molecules. This value is to be compared with the value 1.5×10^{12} from the experiments using a catalase concentration ten times higher.

In solutions with catalase concentration at 1.6×10^{-10} M a complete adsorption will put only about 2×10^{11} molecules on each cm². This number is so much lower than that found at equilibrium in solutions containing salt, that it may account for the lack of effect of adding potassium chloride or buffer.

During reaction with hydrogen peroxide the activity is increased as mentioned above. The velocity constant does not change from about 60 to more than 95 per cent decomposition of hydrogen peroxide, and during this part of the reaction some sort of equilibrium has therefore probably been reached. In that case addition of hydrogen peroxide elutes catalase molecules much more efficiently than addition of salt as only about 1×10^{11} molecules can be left on each cm².

As the number of adsorbed molecules on each cm^2 seems to be independent of the molar concentration of catalase, the activity will be most constant in concentrated solutions, and when the area of glass surface in contact with the solution is small.

Addition of a salt as potassium chloride will shift the equilibrium so that a smaller number of molecules are adsorbed on each cm^2 .

SUMMARY

Dilute solutions of catalase, prepared from horse kidney, will show a decrease in activity upon standing. It is found that this decrease is mainly caused by adsorption of catalase on the glass surface.

The number of catalase molecules carried on each cm^2 seems to be independent of the concentration of catalase. It is lower in dilute salt solutions than in pure water.

The cross section of the catalase molecule is calculated to be about 2 000 square Å.

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