2. Comparative Researches in the Stabilities of Plant Catalase and Animal Catalase[1]

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I. INTRODUCTION

Formerly, Prof. Dr. Kondo, Yonezawa, Chiba and Kawai[2] succeeded in comprehending many important natures of the green leaf enzyme in general after they had minutely and repeatedly studied on the plant carbonic anhydrase which had been regarded as one of the most unstable sorts of green leaf enzyme and accordingly one of the most difficult enzyme to be isolated.

Especially we should note that they found out the plant carbonic anhydrase was perfectly stable in 0.1 M NaCl solution though it was extremely unstable in water.

This finding must have greatly contributed to open the way for their success in isolating the plant carbonic anhydrase from a green leaf.

Since 1939, we have minutely studied the prosperity and decline of catalase in the vegetable world, and during these years we have clarified various natures of catalase, tried to isolate as active catalase as possible, established by our experiments on various plants that plant catalase cooperates with chlorophyll and vitamins very closely to play an important role in the operation of photosynthesis, and found out many interesting facts about the optimum temperature of catalase activity.[3]

Though indeed the enzyme chemistry has made great prog ess ever since, we have found the researches in green leaf enzyme have been conducted very rarely, and notwithstanding that deep and thorough studies have been made on animal catalase, the researches in plant catalase have made very slow progress.

We think this may be due to the fact that students have not often experimented on green leaves to study on plant catalase, as green leaf enzyme is very unstable in water and when the enzyme solution is diluted with water or dialysed against water, the enzyme activity declines conspicuously, and green leaf tissues have never been regarded as a good sample for the studies on enzyme.

As we have recently got some results of our researches in the stabilizer of plant catalase, we report the gist of our study in the following, hoping to receive comments and criticisms of the readers.

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II. EXPERIMENTAL RESULTS

We determind catalase activities by the iodometry [4] in all cases.

We have got the results shown in Fig. 1, comparing the prosperity and decline of activities of animal catalase (beef liver catalase) as time goes by, with those of plant catalase (rice-plant green leaf catalase).

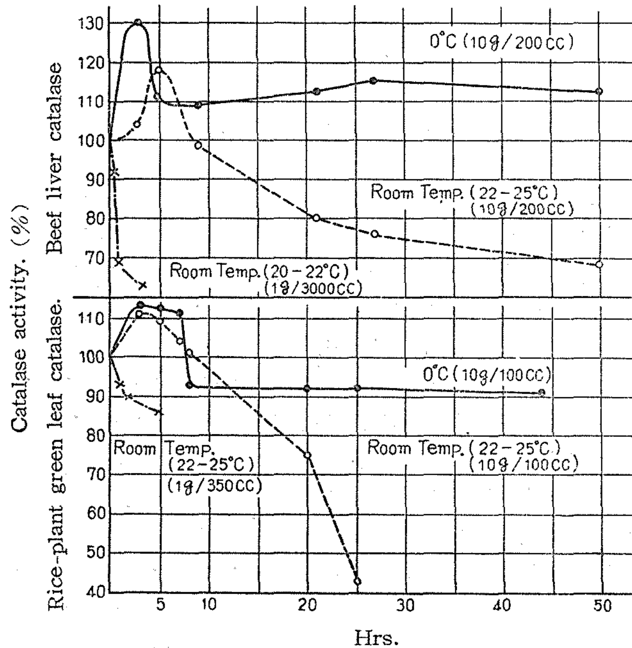

Fig. 1. Comparison of the stability of the solution of animal catalase with that of plant catalase.

The stability naturally differs according to the dilution.

Our dilution of the solution of the beef liver was 10 g/200 cc, and it was half as dense as that of the rice-plant green leaf (10 g/100 cc); however, as indicated by the two curv lines showing the stabilities of their catalase activities at the room temperature in Fig. 1, plant catalase is obviously more unstable.

When we make the dilutions to such a degree as 1 g/3000 cc, the catalase activity is inactivated in a few hours.

As we show in Table 1, we compared the stability of the rice-plant green leaf catalase in the solution of 0.5 M phosphate buffer with each of its stabilities in the solution of 0.5 M NaCl, 0.5 M KCl, 0.5 M $MgSO_4$, and 0.5 M $NaHCO_3$, and we found that the catalase was much more stable in the solution of 0.5 M phosphate buffer and 0.5 M $NaHCO_3$ than in that of 0.5 M NaCl, 0.5 M KCl and 0.5 M $MgSO_4$.

In these experiments, we extracted the catalase from the samples with distilled water, and added each kind of solution of the above-mentioned salts to this water-

Table 1. Experiments on the stabilizers of the rice-plant green leaf catalase (I).

Densities of salts	Catalase activity		
	Initial k	After 6 hrs. k	After 24 hrs. k
0.5M Phosphate buffer (pH=7)	0.0425 (100%)	0.0393 (92.5%)	0.0377 (88.7%)
0.5M NaCl	〃	0.0098 (23.0%)	0.0064 (15.2%)
0.5M KCl	〃	0.0073 (17.2%)	0.00509 (12.0%)
0.5M $MgSO_4$	〃	0.0334 (78.7%)	0.0094 (22.1%)
0.5M $NaHCO_3$	〃	0.0423 (99.6%)	0.0377 (88.7%)
H_2O	〃	0.0316 (74.4%)	0.00857 (20.1%)

Catalase Activity: $k = \frac{1}{t} \log \frac{a}{(a-x)}$

k: velocity constant of monomolecular reaction.
a: titrimetric value of 0.02 N $Na_2S_2O_3$ at zero minute of reaction.
$(a-x)$: titrimetric value of 0.02 N $Na_2S_2O_3$ after a fixed time of reaction.
t: time of reaction (0-2-4-6-8-minutes).

extracted catalase solution and compared the stabilities in each of those cases. Then we extracted catalase from the tissues of green leaves with the solution of the above mentioned salts at such densities as 0.01, 0.05, 0.1 and 0.5 M, and compared the stabilities of the catalase in each of the cases.

As we showed the results in Table 2, we testified 0.01 M phosphate buffer was a very suitable stabilizer.

In order to compare the stabilities of the catalase activities in the same degree, i. e., the stabilities of the animal catalase activity and of the plant catalase activity of the same dilution, we took hen liver catalase and rice-plant green leaf catalase as our samples, and we extracted them respectively with distilled water and 0.01 M phosphate buffer (pH 7.0), and kept them still at room temperature of 18–22° C.

Thus we examined the prosperity and decline of their catalase activities and gave the results in Figs 2 and 3.

We understood clearly that 0.01 M phosphate buffer could effectively be used as a stabilizer.

As you see in Figs 1 and 3, animal catalase activities came, at a time, up to 130 % compared with the initial activities of 100 % just after the extraction of their catalase, and we deem it due to the autolyse.

Table 2. Experiments on the stabilizers of the rice-plant green leaf catalase (II).

Densities of salts	Catalase activity		
	Initial k	After 6 hrs. k	After 24 hrs. k
0.01M Phosphate buffer (pH=7)	0.0505 (100 %)	0.0604 (119.6 %)	0.0514 (101.8 %)
0.05M 〃	〃	0.0548 (108.5 %)	0.0457 (90.5 %)
0.1 M 〃	〃	0.0541 (107.1 %)	0.0447 (88.5 %)
0.5 M 〃	〃	0.0469 (92.9 %)	0.0402 (79.6 %)
0.01M NaCl	0.0464 (100 %)	0.0314 (67.7 %)	0.0118 (25.4 %)
0.05M 〃	〃	0.0236 (50.8 %)	0.0131 (28.2 %)
0.1 M 〃	〃	0.0216 (46.6 %)	0.0111 (23.9 %)
0.5 M 〃	〃	0.0105 (22.6 %)	0.0065 (14.0 %)
0.01M $NaHCO_3$	〃	0.0488 (105.2 %)	0.0386 (83.2 %)
0.05M 〃	〃	0.0496 (106.9 %)	0.0368 (79.3 %)
0.1 M 〃	〃	0.0455 (98.1 %)	0.0390 (84.1 %)
0.5 M 〃	〃	0.0408 (88.0 %)	0.0302 (65.1 %)
H_2O	0.0505 (100 %)	0.0305 (65.7 %)	0.0043 (9.2 %)

Set at room temperature (18°-20°C) Dilution: 1 g/300 cc

Table 3. Effect of dialysis.

	Initial activity k	After 48 hrs. k	Survived %
0.01M Na_2HPO_4	0.0382	0.0356	93.2
0.05M Na_2HPO_4	0.0382	0.0349	91.3
0.01M $NaHCO_3$	0.0382	0.0346	90.6
0.05M $NaHCO_3$	0.0382	0.0337	88.2
0.01M NaCl	0.0382	0.0223	58.4
0.05M NaCl	0.0382	0.0203	53.1
H_2O	0.0382	0.0170	44.5

Sample: green leaf of the rice-plant.
Dilution: 10g/100cc. set at 0~2°C.

Then we examined the effect of dialysis on the animal and plant catalase activities, and as we showed on Table 3, when we dialysed the catalase against water for 48 hours, only 44.5 % of the activities survived. But we found that when we dialysed

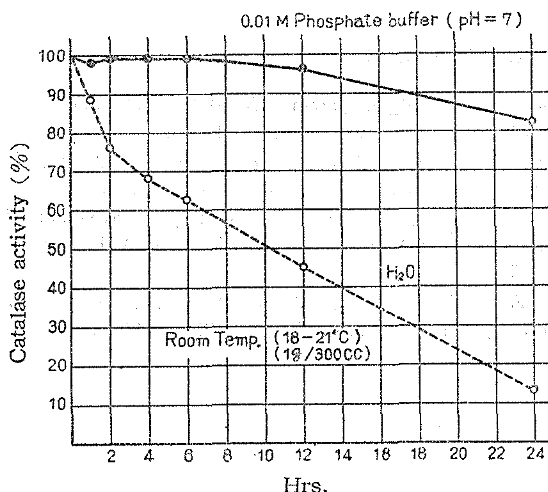

Fig. 2. Experiments on the stabilities of the rice-plant catalase

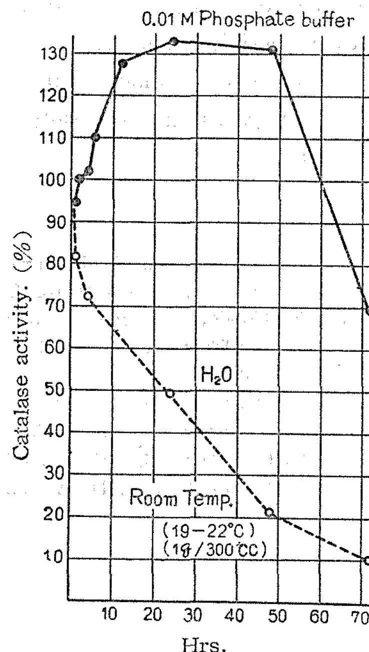

Fig. 3. Experiments on the stabilities of the hen liver catalase.

the catalase against 0.01 M Na_2HPO_4 for 48 hrs., as much as 93.2 % of the activities survived. In case of dialysis, NaCl cannot be used as a stabilizer on catalase.

These data of dialysis against phosphate buffer and $NaHCO_3$ are consistent with what are shown in Tables 1 and 2, and Figs. 2 and 3.

III. SUMMARY

1) We compared the stabilities of the various catalase solutions at 0° C and room temperature (22–25° C).

2) Comparing the stabilities of the rice-plant green leaf catalase in the 0.5 M solutions of phosphate buffer, NaCl, KCl, $MgSO_4$ and $NaHCO_3$, we found that 0.5 M phosphate buffer and 0.5 M $NaHCO_3$ could be used as stabilizers.

3) Extracting catalase from the tissues of green leaves with various kinds of solution of the above-mentioned salts at the densities of 0.01, 0.05, 0.1, 0.5 M, we testified by the comparison that 0.01 M phosphate buffer was a very suitable stabilizer.

4) Examining the prosperity and decline of the animal and plant catalase activities at the same dilution of the distilled water and 0.01 M phosphate buffer kept at the room temperature (18–22° C), we confirmed that though the plant catalase was much more unstable in the water-extracted solution than the animal catalase, 0.01 M phosphate buffer could be used as a very suitable stabilizer commonly to the animal catalase and the plant one.

(5 We found that when dialysed against water for 48 hrs, only 44.5 % of the catalase activities survived, but when dialysed against 0.01 M phosphate for the same length of time, as much as 93.2 % of the activities could survive.
6) We hope the above results will be of much benefit to the isolation of the plant catalase.

IV. REFERENCES

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