COMPARATIVE STUDIES ON THE CATALATIC REACTION BETWEEN RED CELL SUSPENSION AND HEMOLYSATE

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We have very often observed that a cell or a tissue has a different biophysico-chemical activity from that of its homogenate. For instance, as is mentioned in the previous report (1), in red cell suspension no spontaneous deactivation of catalase occurs so long as there is no hemolysis, while a marked deactivation takes place spontaneously in hemolysate. In the present study, the difference in reaction type of catalase between red cell suspension and hemolysate was investigated.

MATERIAL AND METHOD

Catalatic reactions of diluted blood of various vertebrates were investigated by the thermoanalytical method (1). Fifty ml. of the diluted blood (red cell suspension or hemolysate) prepared in the same manner as described in the previous report (1) and 50 ml. of 0.05 M H_2O_2 solution with or without 0.16 M phosphate buffer, were made to react in the calorimeter. Temperature-change in the reaction system, ΔT , was measured with time, t , and analysed. From the thermoanalytical equation,

$$\frac{dT}{dt} + K\Delta T = \frac{Q}{W} \frac{dx}{dt},$$

K : the cooling constant of the reaction system

W : the water equivalent of the reaction system.

Q : the decomposition heat of H_2O_2 .

the reaction velocity, $\frac{dx}{dt}$, is obtained. As the main part of the reaction is of the first order, the reaction velocity constant, k_1 , is also obtained from the slope of the straight part of the $\log \frac{Q}{W} \frac{dx}{dt} \sim t$ curve. The temperature of the experiment is always 25°C. In this paper, the catalase activity or the velocity constant of the first order reaction, k_1 , is expressed in reciprocal minutes.

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RESULTS

Fig. 1 and fig. 2 demonstrate the reactions by various concentrations of human hemolysate and red cell suspension, respectively. The $\Delta T \sim t$ curve of hemolysate (fig. 1) is apparently a smooth, hyperbolic curve, while, in case of red cell suspension (fig. 2) especially of low concentrations, the $\Delta T \sim t$ curves, take a slight S-shape toward the end of the reaction, showing an inflection point. Furthermore, as is evident from a $\log \frac{Q}{W} \frac{dx}{dt} \sim t$ curve, in case of hemolysate, especially of low concentrations, the final part of the curve deviates from a straight part, bends upwards; that is, the retarded stage (2) (what Williams (3) call induced inactivation) appears, deviating from the main first order reaction. On the contrary in red cell suspension the reaction velocity is accelerated following the 1st order reaction as indicated by arrows in fig. 2 *b*.

The reaction with red cell suspension, that with hemolysate and changes in the rate of reaction by using blood catalase once affected by H_2O_2 are comparatively shown in fig. 3. Human blood was used in all these observations. By comparing the velocity constant of catalytic reaction between red cell suspension and hemolysate, one may notice that the velocity constant is a few times larger in hemolysate than in red cell suspension in spite of both containing the same amount of catalase. When a certain amount of H_2O_2 has been added to the test solution before using it for the observation, red cell suspension shows a much larger reaction velocity compared with that in ordinary red cell suspension, while the velocity is reduced in hemolysate. The activity of catalase of hemolysate becomes weaker progressively in proportion to the concentration of H_2O_2 previously used.

DISCUSSION

Of the above findings the important point to consider is why the rate of catalytic reaction of red cell suspension is increased when the red cell suspension has been treated beforehand with H_2O_2 . The catalase molecules in the red cell cannot be supposed to be activated by H_2O_2 , neither the cell is hemolysed by it. The author has previously found (1) that the apparent activation energy for catalytic reaction is 7.2 Cal. in red cell suspension and 13.9 Cal. in hemolysate. Considering that the rate of reaction in hemolysate indicates directly the rate of decomposition of H_2O_2 , the smaller rate in red cell suspension must depend upon some other factor, very probably the rate of diffusion of H_2O_2 into the cell. Coincidentally with this consideration, we noticed that the reaction in red cell suspension was considerably accelerated by stirring the reaction system (4, 5).

Granted that the above assumption is true, we may conclude that the structure of the cell membrane is changed by the effect of H_2O_2 in the sense of increase in its permeability. Concomitantly with this change, the red cell is fixed so that its hemolysis no longer occurs by the effect of distilled water, saponin, etc. as will be described in the next paper.

In the previous paper (2), the author reported that the blood catalase reaction in hemolysate develops in three stages—the initial stage, steady stage (the 1st order) and retarded stage. With red cell suspension, however, the last

FIG. 1a

FIG. 1b

FIG. 1. Reaction between hemolysates in which human blood is diluted 1,000-5,000 times with 0.016 M phosphate buffer (pH 6.8) and 0.05 M hydrogen peroxide solution.

FIG. 2. Reaction between red cell suspensions in which human blood is diluted 100-1,000 times with 0.16 M phosphate buffer (pH 6.8) and 0.05 M hydrogen peroxide solution containing 0.16 M phosphate buffer.

FIG. 3. Comparison between the catalytic reaction by red cell suspension and that by hemolysate (man). Though the ordinate is displaced for each curve, the scale of both ordinate and abscissa is equal. No. 1: Reaction by red cell suspension (1:1,000 diluted blood with 0.16 M phosphate buffer). No. 2: Reaction by 1:1,000 diluted red cell suspension prepared by adding 25 ml. of 0.05 M H_2O_2 solution containing 0.16 M phosphate buffer to 25 ml. of 1:500 diluted blood with 0.16 M phosphate buffer. No. 3: Reaction between the hemolysate containing 0.16 M phosphate buffer (dilution of blood, 1:1,000) and H_2O_2 solution containing 0.16 M phosphate buffer. No. 4: Reaction by the hemolysate containing 0.016 M phosphate buffer (dilution of blood, 1:1,000). No. 5: Reaction by the hemolysate (dilution of blood, 1:1,000) prepared by adding 25 ml. of 0.05 M H_2O_2 solution to 25 ml. of hemolysate containing 0.032 M phosphate buffer (dilution of blood, 1:500).

stage does not appear as shown in fig. 2*b*. This is probably so because the retarded phenomenon is counteracted by acceleration due to increase in permeability above mentioned. In the character of action of catalase there seems to be no difference whether it is in the cell or dissolved in the plasma.

SUMMARY

1. The rate constant of the catalytic reaction of hemolysate is only a few times as large as that of the catalytic reaction of red cell suspension.

2. The activity of hemolysate catalase once treated with H_2O_2 is reduced in proportion to the concentrations of H_2O_2 previously added. On the contrary, the velocity of the catalytic reaction of red cell suspension once treated with H_2O_2 is a few times as large as that in the original red cell suspension.

3. When the amount of red cell is small in both red cell suspension and hemolysate, a retarded stage appears in their catalytic reactions, but in case of red cell suspension, the acceleration of hydrogen peroxide decomposition appears at the same time.

4. The rate of catalytic reaction in red cell suspension seems to be determined by the rate of diffusion, which may be increased by H_2O_2 .

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