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Abstract

For the purpose to clarify further the residual catalase in the blood cell ghost, the ghost has been applied to Cyanogum and starch block electrophoresis and the results are briefly summarized as follows. 1. It has been demonstrated that after Cyanogum electrophoresis of the ghost after several washings, bubbling due to enzymatic reaction of catalase occurs near the points of origin, when the plate is immersed in hydrogen peroxide solution and also it has been proved the presence of catalase so firmly bound to the ghost that it is hardly moved by the electro phoresis. Even with the ghost of hypocatlasemia there can be detected catalase which is likewise hardly eluted from the ghost. 2. In the estimation of catalase activities of each fraction from red cell ghost by starch block electrophoresis there can be detected catalase near the point of origin, that is not eluted by the electrophoresis, and the activity of which corresponds to about 0.1 % of the total red cell catalase activity.

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CATALASE ACTIVITY IN THE RED CELL GHOST OF HYPOCATALASEMIA AND NORMAL SUBJECT
II. ISOLATION OF CATALASE IN THE RED CELL GHOST BY ELECTROPHORESIS

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In a previous paper the author reported that catalase in the red cell ghost maintains its activity at a certain level even after repeated washings of the ghost. In addition, having found catalase activity in the supernatant after each washing under fixed conditions, it was reasoned that theoretically the catalase in the red cell can be classified into two kinds; namely, one with stronger affinity to the ghost and the other with less affinity, hence more readily liberated from the ghost. In the present experiments the author conducted the Cyanogum electrophoresis of the washed ghost, hemolysate and red cell catalase extract, and compared the obtained results. Further, the starch block zone electrophoresis of the ghost was carried out in order to find out the percentage of the stroma-bound catalase activity to that of the whole blood catalase as well as the percentage of catalase released from the ghost during washing. Some outstanding data of the study are presented in this report.

MATERIALS AND METHODS

Materials: Fresh bloods from normal persons and hypocatalasemic subjects served as materials. Special care was taken in selection of the subjects and the blood was aspirated from the cubital vein of healthy persons without disease.

Methods:

1. The ghost solutions were all prepared by the following methods.

a) The ghost solution of normal persons: After washing normal human blood cells three times with cold physiological saline solution, the blood cell sediment is hemolyzed against 50 volumes of deionized water, then washed 3 times with CO_2-saturated water, homogenized with Potter-Elvehjem glass homogenizer, and homogenates so prepared served for the experiments.

b) Ghost solutions of hypocatalasemia: Blood is washed 3 times with cold physiological saline solution, the blood cell sediment is hemolyzed against 5 volumes of deionized water, centrifuged, again washed with 5 volumes of deioni-

zed water, centrifugations are repeated 4 times, and the final sediment is homogenized to be used in the experiments.

2. The method of estimating catalase activity

Just as in the previous reports, catalase activity was determined by a modification of EULER-JOSEPHSON's potassium permanganate method (1, 2).

3. Cyanogum electrophoresis

After filling the Cyanogum-41 plate (3) with each of the ghost solutions, hemolysate or blood catalase extract in a cold room (4°C), and with Tris solution, EDTA, boric acid (pH 8.9) as buffer according to PAULICK (4) under the conditions of: 1.2 mA current for 1 cm width, and the electrophoresis is carried out for 15 hours. After the run the plate is immersed in 1.0% H_2O_2 solution to examine the catalase reaction, then stained with 0.1N potassium permanganate, and decoloration with 5% acetic acid is carried on for 30 minutes to confirm catalase. For comparison, the hemolysate simultaneously was subjected to the electrophoresis, For preparing the above hemolysate, sediment is precipitated by centrifugation and the blood sediment thus obtained is hemolyzed against deionized water of the volume equal to that of original blood. The extract of blood catalase is prepared as follows; red cells are washed 3 times with cold physiological saline solution, centrifuged. The red cell sediment is hemolyzed against deionized water of the volume equal to the original blood, to this 0.42 volume of ethanol-chloroform (3 : 1, v/v) mixture is dripped little by little, centrifuged after adding an equal volume of acetone, then to the sediment is added 0.1M acetate buffer (pH 5.6) until the sediment is dissolved.

4. Starch block electrophoresis of horizontal type (5)

The two boxes of synthetic resin measuring 0.2 cm thick, 1.5 cm high, 3 cm wide and 40 cm long, are filled with the mixture of starch powder as the supporting material and buffered (the same as used in Cyanogum electrophoresis), at each starting point V-shaped furrow is made, then the ghost and hemolysate (from which substrate was removed by centrifugation) are poured into each furrow, and the electrophoresis is run in a cold room. The conditions for electrophoresis are: 2 mA/cm^2 at 200V for 15 hr. For the buffer solutions, Tris, EDTA, boric acid (pH 8.9) are also used according to PAULIK (4). After the electrophoresis the starch block is cut into pieces of 1 cm in width, to the piece is added 2 ml buffer solution (the same as used in electrophoresis), further 1 ml of 0.2 mM deoxycholate is added, stirred well, left standing for 15—30 min., centrifuged to prepare the supernatant, and with the extract so obtained catalase activity is estimated. In the preliminary experiments conducted prior to the experiments, it has been confirmed that deoxycholate has no substantial effect on the activity of catalase contained in the ghost, alteration being within the range of experimental error.

RESULTS

As is evident from the results (Fig. 1), in the test with 1.0% H_2O_2 aqueous solution of Cyanogum-41 plate after the electrophoresis near the starting

point slightly closer to the anode there can be observed vigorous gas bubbling due to enzymatic reaction of catalase by hydrogen peroxide. At the same time this photograph shows spots where gas bubbles have evolved,

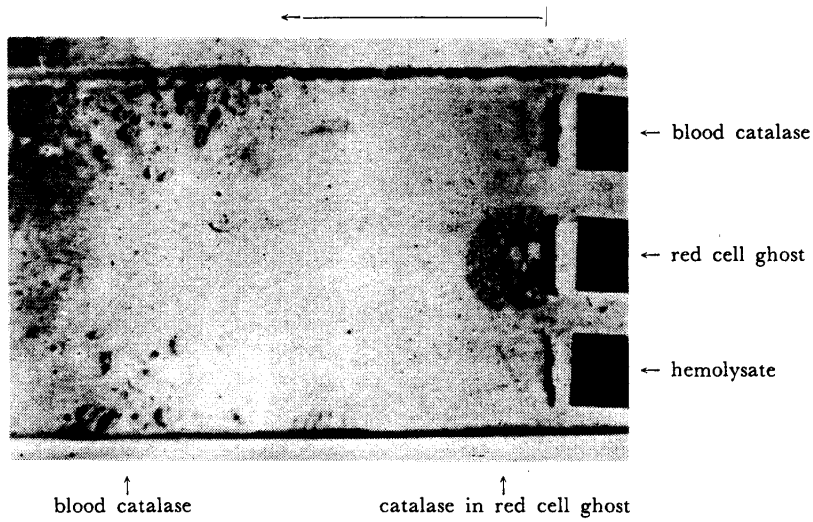

Fig. 1. Showing the catalase activity of the red cell ghost after Cyanogum electrophoresis
The plate after electrophoresis was steeped in 1.0% H_2O_2 solution and bubblings show the catalase activity present

implying the presence of purified blood catalase and hemolysate catalase activities. As for these spots in every spot centering around the point 5 cm from the origin close to the anode there is observable bubble evolution band 2 cm in width. In other words, catalase of hemolysate is detectable just as readily as blood catalase extract. This finding differs distinctly from the finding in the electrophoresis of red cell ghost where bubbles can be seen only in the vicinity of the origin. Likewise in the electrophoresis of hypocatalasemic red cell ghost bubbles can be observed only near the origin.

The results of the starch block electrophoresis of the ghost as well as the hemolysate removed of the ghost are shown in Fig. 2. In the electrophoresis of the ghost there can be seen the presence of catalase near the origin ($+2 \sim -3$) which does not migrate while there is another migrating catalase, though minute in quantity, located close to the cathode at the site corresponding to free catalase. As for the supernatant of hemolysate removed of its ghost and run simultaneously, a high peak of catalase activity is observed at the site about 8 to 12 cm from the origin towards the cathode but there is not any activity observable near the origin. It has

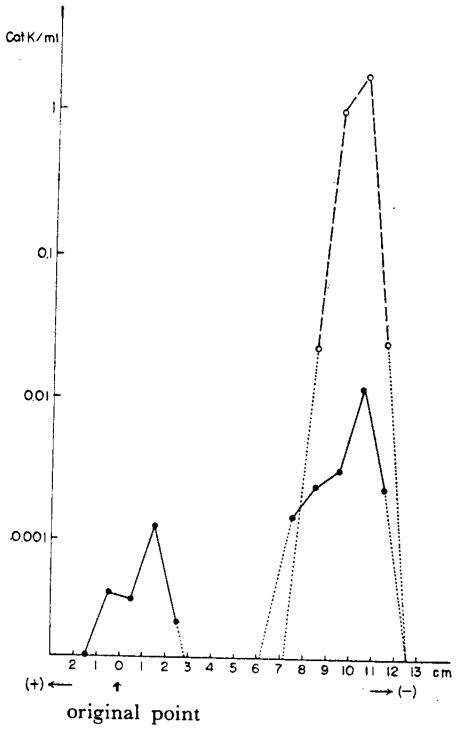

Fig. 2. Catalase activities of fractions isolated by starch block electrophoresis
●——● red cell ghost
○---○ supernatant of hemolysate

been confirmed that this peak represents free catalase of hemolysate.

Looking at the extent of catalase activity of each fraction isolated, the total catalase activity (Cat K) of the red cell ghost around the origin in the electrophoresis has been found to be 3.499×10^{-3}, that between 7 and 10 cm towards the cathode 25.01×10^{-3}, and the total catalase activity of hemolysate between 8 and 12 cm close to cathode 3.640 (each value calculated per unit of 1 ml of blood used). In other words, by a rough estimation the percentage of the total residual catalase activity near the origin as against the blood cell catatase turns out to be 0.1%. Furthermore, the percentage of the catalase which has been released from the red cell ghost amounts to 0.7 %.

DISCUSSION

Mobility of the red cell catalase during the Cyanogum electrophoresis of the ghost

Concerning the electrophoresis of hemoglobin of red cell ghost,

KLIPSTEIN (6) has reported about its specificity, and PONDER (7) has recognized hemoglobin in the red cell ghost to remain at the origin during the paper electrophoresis. However, there seems to be no report concerned with electrophoresis of blood catalase in the ghost. The evolution of gas bubbles at the origin in Cyanogum electrophoresis has demonstrated the enzymatic reaction of catalase, and it is assumed that catalase is contained in the red cell ghost in a state not to be eluted by electrophoresis. Migration (mobility) at the site of this bubble evolution does not differ appreciably between blood catalase of normal person and that of hypocatalasemic individual, and it seems that the ghost of hypocatalsemia contains catalase likewise in a firmly bound state.

Isolation of catalase in the red cell ghost by starch block electrophoresis

From the results of the previous as well as the present experiments of Cyanogum electrophoresis the author has verified that there exists a firmly bound stroma catalase, and further has demonstrated by the quantitative analysis of the catalase activity of each fraction that at least 0.1% of the total red cell catalase activity is contained in the ghost. This point has also been assumed in the previous report, and even from the quantity of hemoglobin in the ghost it is possible that still a higher concentration of catalase might be contained. In addition, the catalase content in the ghost seems to be dependent upon the conditions of hemolysis and washing. As clearly shown in Fig. 2, the electrophoresis of the ghost after 3 washings there can be detected some free catalase which indicates that some catalase is liberated at each washing, though only in a minute quantity, while on the other hand, there exists also catalase firmly bound to the ghost (stroma-bound catalase). With respect to the residual catalase in the ghost, it seems to differ from free catalase of the red cell in various properties, and it requires further study, and the action mechanism of the ghost catalase that operates *in vivo* is also a problem to be clarified.

CONCLUSION

For the purpose to clarify further the residual catalase in the blood cell ghost, the ghost has been applied to Cyanogum and starch block electrophoresis and the results are briefly summarized as follows.

1. It has been demonstrated that after Cyanogum electrophoresis of the ghost after several washings, bubbling due to enzymatic reaction of catalase occurs near the points of origin, when the plate is immersed in hydrogen peroxide solution and also it has been proved the presence of catalase so firmly bound to the ghost that it is hardly moved by the electro-

phoresis. Even with the ghost of hypocatlasemia there can be detected catalase which is likewise hardly eluted from the ghost.

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