Residual Catalase Like Protein in the Erythrocytes of Japanese Acatalasemia

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Immunoelectrophoresis was carried out on the crude catalase from the erythrocytes of the patient with acatalasemia against antihuman erythrocyte catalase rabbit serum and the presence of residual catalase like protein was observed in the erythrocytes of Japanese acatalasemia.

Presence of residual catalase activity in the erythrocytes of Japanese acatalasemia was reported in the previous report (Ogata et al. 1972). Aebi et al. (1964) found the presence of the residual catalase like protein in the blood of Swiss acatalasemia by the double diffusion method between crude catalase fraction and antihuman blood catalase rabbit serum. Recently, Nishimura et al. (1968) reported that the three-band pattern was observed in the reaction of the lysate of erythrocytes from normal person against antihuman erythrocyte catalase rabbit serum in immunoelectrophoresis. This report deals with the results obtained by the immunoelectrophoresis on the crude catalase fraction from acatalasemia erythrocytes against antihuman erythrocyte catalase rabbit serum.

Materials and Methods

Fresh human blood of the normal and the patient with acatalasemia were taken from the antecubital vein with heparine. The acatalasemia blood samples (GI-, and NA-family) employed in this study were obtained from individuals whose genotypic and phenotypic characterization of the biochemical defect have been studied throughly.

Purified normal erythrocyte catalase (PNEC), stage 5 of Herbert and Pinsent (1948), having a kat. f of 60,000, was prepared from the pooled normal human blood. After centrifugation, plasma and buffy coat were aspirated and discarded. The remaining erythrocytes were washed three times with saline and hemolyzed. The hemolysate was applied to the DEAE column (1.5 x 21 cm) (Ogata et al. 1966). Elution of catalase like protein from the column was conducted with a series of sodium phosphate buffer (pH 6.8) of different concentrations: 1 mM, 3 mM and 0.1 M. The catalase activity was found to be present in the elution of 0.1 M buffer by the perborate method. Repeating these chromatographic procedures several times, about 150 ml of catalase like fraction was taken and concentrated to 1 ml by collodion bag. The condensed catalase like protein thus obtained, i.e. crude acatalasemia erythrocyte catalase (CAEC), was used to immunoelectrophoresis as antigen. All procedures were conducted in the cold room controlled at 6°C.

To induce antibody, PNEC was emulsified with Freund’s complete adjuvant so that the final concentration was to be 1.0 mg/ml and a rabbit received an injection of 3.5 ml of the PNEC suspension subcutaneously once. Twice “booster” inoculation of the aqueous enzyme preparation containing 0.1 mg of catalase was performed intravenously every other day, 2 weeks after the first immunization.

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Immunoelectrophoresis employing 1.0% agar in barbital buffer of 0.05 ionic strength and a pH of 8.4 was carried out at a constant voltage of 100 V for 1 hour. The precipitin reaction was developed in a cold room for 2–3 days.

**RESULTS**

Immunoelectrophoretic studies using the PNEC and CAEC against anti PNEC were conducted. As shown in Fig. 1, two- or three-band patterns were characteristically observed in CAEC of two acatalasemias, one of which correspond to the band obtained from PNEC. It is thus concluded that the electrophoretic mobility of catalase like material obtained from CAEC is identical with that of PNEC. In the preliminary experiments, it has not been possible to detect any catalase protein in a total hemolysate of acatalasemia blood by immunoelectrophoresis. However, after concentrated 150 fold with DEAE column chromatography, the material reacting with an antiserum specific for catalase could be demonstrated to be present in acatalasemia erythrocytes.

![Diagram](image)

**DISCUSSION**

In the previous study, separation of residual catalase protein with Sephadex G-100 column chromatography was carried out on acatalasemia hemolysates. It was found that Michaelis constants of normal and residual catalase activity were 0.35 and 0.37 mM, respectively (Ogata et al. 1972). The residual catalase molecules were suggested to be present in acatalasemia erythrocytes. In the present immunological experiments, data indicated the presence of catalase like protein in the erythrocytes of Japanese acatalasemia.

**References**