Fractionation of Erythrocyte Catalase from Normal, Hypocatalatic and Acatalatic Humans*

S. MATSUBARA, H. SUTER, and H. AEBI
Medizinisch-chemisches Institut, Universität Bern

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1. By column chromatography on DEAE-cellulose calcium phosphate mixed gel red cell catalase can be separated in two fractions, 0.15 M saline and 0.15 M secondary phosphate being used as eluants.

2. The fractions obtained from blood of normal subjects and of homozygous acatalasia cases differ in electrophoretic mobility and in heat stability. Of the fractions isolated from blood of heterozygous cases, one resembles electrophoretically the normal and the other the acatalatic enzyme.

3. The observation that the enzyme fractions isolated from acatalatic blood are less stable and are mainly localized in reticulocytes, suggests, that in acatalasia a labile enzyme variant is synthesized. Therefore, acatalasia may be considered as an enzyme defect due to a structural gene mutation.

Human red cell catalase and rat liver catalase have been reported recently to exhibit heterogeneity [3, 4]. Therefore it seemed to be of interest to investigate further the chemical nature of catalase activity detectable in small amounts in homozygous cases of acatalasia (about 1% of normal level) and to compare its properties with those of the enzyme isolated from normal human blood. Catalase active material isolated previously from normal and acatalatic human red cells by gel filtration proved to be identical in respect to mobility in gel filtration, azide sensitivity and precipitation by anticatalase [5, 6]. This led to the assumption that acatalasia — at least in the Swiss cases — may be considered as a controller gene mutation [7]. However, it could be demonstrated, that, by applying a separation technique similar to that devised by TRÖLLE et al. [3] fractions of different electrophoretic mobility can be distinguished in blood of heterozygous cases of acatalasia [8].

In earlier experiments gel filtration was used in order to achieve a rapid one-step separation of total red cell catalase from the hemoglobin moiety [9].

* This is contribution No. 3 of a series of papers on acatalasia published in this journal [1, 2].
For this study, however, requiring larger quantities of material, an alternative technique was devised. By means of column chromatography on calcium phosphate-DEAE-cellulose complex gel catalase can be separated from hemoglobin in one single step as well. In addition two fractions of catalase can be obtained by subsequent extraction of the column with 0.15 M sodium chloride solution (= fractions with index number 1) and with 0.15 M secondary sodium phosphate solution pH 8.2 (= fractions with index number 2). This separation technique has been applied to samples of (a) normal human blood, (b) blood of a heterozygous and (c) blood of a homozygous case of acatalasia. Thus six different preparations of purified human red cell catalase are obtained.

This report deals with the technique of red cell catalase fractionation. Furthermore some properties of the fractions in respect to electrophoretic mobility, heat stability, antigenic identity and sedimentation velocity are described. The results indicate that both fractions obtained from blood of homozygous acatalas(em)ia cases and one of the two fractions obtained from blood of heterozygotes (“Hypocatalas(em)ia”) differ from those isolated from normal human blood at least what their electrophoretic mobility and heat stability are concerned. These results, together with those given in earlier reports [2, 5, 6] shall serve as an experimental base for a discussion of the alternative whether the acatalatic condition (acatalas(em)ia) has to be considered as a controller gene- or a structural gene mutation.

Methods

1. Preparation of DEAE-Cellulose Calcium Phosphate Gel

50 g (wet weight) of DEAE-cellulose were suspended in 250 ml of distilled water. To this suspension 750 ml of 0.10 M calcium chloride (11.03 g CaCl₂ · 2H₂O) solution and, under continuous stirring, 375 ml of 0.15 M sodium triphosphate (21.38 g Na₃PO₄ · 12H₂O) solution were added. The mixture was first stirred for 5 hours at room temperature and then for 24 hours at 5°C. The mixture became slightly thick and was left overnight at 5°C. The resulting sediment was washed repeatedly by decantation until an almost clear supernatant was obtained. Finally, it was put on a funnel, the residue suspended in distilled water and adjusted to pH 7.0 with 0.02 M potassium dihydrogen phosphate solution. The sediment was washed again several times with distilled water and filtered. The gel was stored before use at 5°C in approximately 2 liters of distilled water.

2. Column Chromatography of Hemolysate on DEAE-Cellulose Calcium Phosphate Gel

3.5 ml of washed erythrocytes were laked by addition of twice the volume of distilled water; the hemolysate was dialysed against water for 2 days and finally filtered with super-celite. About 10 ml of the hemolysate were applied to a DEAE-cellulose-calcium phosphate gel column (40×1.3 cm). The column was first washed with distilled water until the eluate became colorless; the elution was carried out with 0.15 M saline, and then with phosphate buffer of stepwisely increasing concentration and pH. The gradient was obtained by using 150 ml of 0.02 M, pH 5.7, and 150 ml of the same buffer 0.2 M, pH 7.0 in the mixing flask. The flow rate was 3 to 4 ml per hour; the effluent was collected in 3 ml fractions.