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HUMAN ERYTHROCYTE ACETYLCHOLINESTERASE

II. EVIDENCE FOR THE MODIFICATION OF THE ENZYME BY ION-EXCHANGE CHROMATOGRAPHY

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SUMMARY

1. Triton-solubilized acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7) eluted from a column of DEAE–Sephadex with one-step salt elution revealed a single electrophoretic acetylcholinesterase component (acetylcholinesterase-3), intermediate between the previously described components of acetylcholinesterase-1 and acetylcholinesterase-2. When the column of DEAE-Sephadex was eluted with a linear salt gradient, the area between the two peaks of enzymatic activity contained only one component with the electrophoretic mobility of acetylcholinesterase-3.

2. It is postulated that the Triton-solubilized acetylcholinesterase is a hybrid dimer composed of two unlike components (α and β) of equal size.

The resolution of human erythrocyte acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7) by ion-exchange chromatography into two components of apparently identical molecular weight was previously reported[1].

The present communication describes further studies on these components and presents evidence that these acetylcholinesterases may have resulted from the modification of the Triton-solubilized enzyme by ion-exchange chromatography.

Heparinized blood (20–40 ml) was obtained from human volunteers. Blood samples from different donors were processed separately.

Triton-solubilized acetylcholinesterase was prepared as previously described[1]. Acetylcholinesterase activity was determined colorimetrically[2] using a Beckman DU spectrophotometer equipped with a recorder. Cellulose acetate gel electrophoresis, agarose (Sepharose 4B) gel filtration and DEAE-Sephadex column chromatography were performed as previously described[1]. In some experiments following the application of the Triton-solubilized acetylcholinesterase, the column of DEAE-Sephadex was eluted with 100 ml of 0.5 M NaCl (in phosphate buffer*, 0.05% Triton X-100) instead of the application of a linear salt gradient. The appropriate fractions were dialyzed

* All phosphate buffers had a pH of 8.0 (0.01 M) and were 1 mM in EDTA and 2-mercaptoethanol.

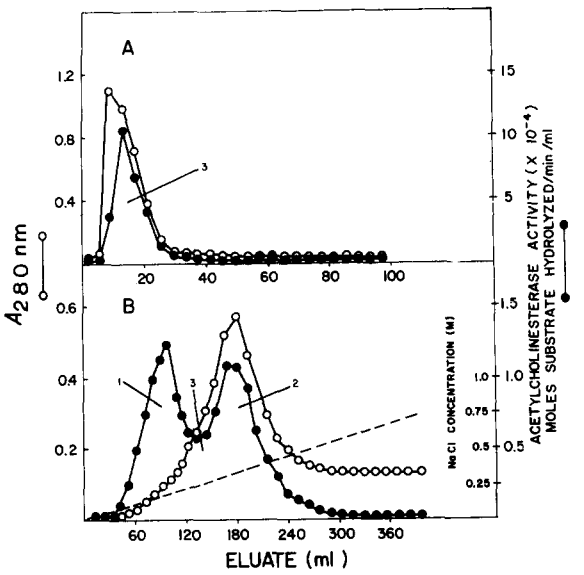

Fig. 1. DEAE-Sephadex column chromatography patterns of acetylcholinesterase. A. One-step salt elution: Triton-solubilized acetylcholinesterase preparation was applied to a column of DEAE-Sephadex and eluted as described in the text. The recovery of enzymatic activity was approximately 70%. B. Linear gradient elution: Triton-solubilized acetylcholinesterase preparation was eluted from an identical column of DEAE-Sephadex with a linear salt gradient as previously described[1]. The areas labeled 1, 2 and 3 contain acetylcholinesterase-1, acetylcholinesterase-2 and acetylcholinesterase-3, respectively.

against several changes of phosphate buffer, concentrated in membrane filters of less than 5 nm porosity, and used for electrophoresis as previously described[1].

Acetylcholinesterase was eluted as a single peak from the column of DEAE-Sephadex using one-step salt elution (Fig. 1A) as compared to the two peaks of activity obtained by linear gradient elution as previously reported[1] (Fig. 1B). Aliquots from the ascending, maximum, and the descending segments of the peak obtained by one-step salt elution were shown to have a single electrophoretic acetylcholinesterase component of identical mobility (acetylcholinesterase-3). The mobility of this component was intermediate between the previously described components[1] of acetylcholinesterase-1 and acetylcholinesterase-2 (Fig. 2). Re-chromatography of acetylcholinesterase-3 on a column of DEAE-Sephadex with the application of an NaCl gradient resulted in the resolution of the enzymatic activity into two peaks, identical with the previously described results utilizing Triton-solubilized acetylcholinesterase[1]. In the case of the Triton-solubilized acetylcholinesterase and acetylcholinesterase-3, the area between the two peaks contained only one component with the electrophoretic mobility of acetylcholinesterase-3. It was further shown that the Triton-solubilized acetylcholinesterase eluted from a column of agarose had only one electrophoretic component identical with acetylcholinesterase-3. The three components as well as the Triton-solubilized acetylcholinesterase had an apparent molecular weight of 420 000 by gel filtration technique[1].

The electrophoresis of Triton-solubilized acetylcholinesterase was unsuccessful due to the presence of 2.5% Triton X-100 in the preparation. However, after reducing

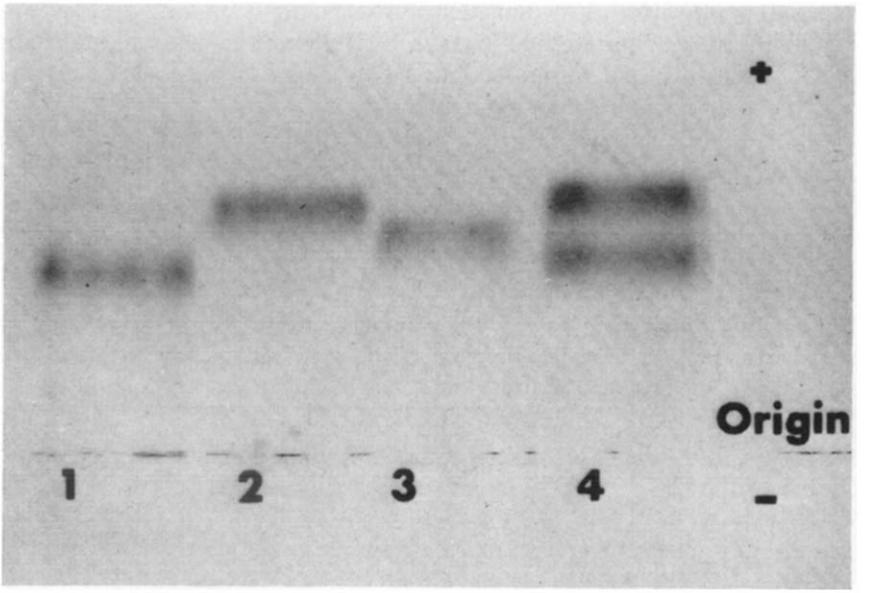

Fig. 2. Cellulose acetate gel electrophoresis of acetylcholinesterase components. Aliquots (2–5 μl) of (from left to right) acetylcholinesterase-1, acetylcholinesterase-2, acetylcholinesterase-3, and a mixture of acetylcholinesterase-1 and acetylcholinesterase-2 were electrophoresed and histochemically stained.

the concentration of the detergent in the agarose column to 0.05%, there was only one electrophoretic component. This component remained intact when the column of DEAE-Sephadex was eluted with 0.5 M NaCl, but it was resolved into three electrophoretically distinct components upon elution of the column with a linear salt gradient. Acetylcholinesterase-1 and acetylcholinesterase-2 remained intact upon rechromatography on DEAE-Sephadex with the application of a salt gradient or one-step salt elution. Mixtures of acetylcholinesterase-1 and acetylcholinesterase-2 did not associate to form the intermediate component of acetylcholinesterase-3. Identical results were obtained using outdated blood from the blood bank.

The foregoing data suggest the dissociation of the Triton-solubilized acetylcholinesterase (acetylcholinesterase-3) on DEAE-Sephadex with subsequent re-association to form the modified acetylcholinesterase-1 and acetylcholinesterase-2. It may be postulated that the Triton-solubilized acetylcholinesterase is a hybrid dimer composed of two unlike components (α and β) of equal size. The Triton-solubilized acetylcholinesterase may dissociate upon binding to DEAE-Sephadex and each component is eluted separately following the application of a linear salt gradient. The dimerization of the like components results in the formation of the modified enzymes, acetylcholinesterase-1 (α_2) and acetylcholinesterase-2 (β_2). The overlapping area between the two peaks contains the hybrid acetylcholinesterase ($\alpha\beta$), since both α and β components are eluted together. The modified enzymes (acetylcholinesterase-1 and acetylcholinesterase-2) may not form the hybrid acetylcholinesterase ($\alpha\beta$) upon mixing since the binding areas have been already occupied. The finding of the hybrid dimers ($\alpha\beta$) following one-step salt elution suggests that the two unlike components, α and β,

may bind preferentially instead of dimerizing to form the α_2 and β_2 components. Therefore, solubilization of the stromal proteins by Triton X-100 may result in the dimerization of the acetylcholinesterase components α and β to form the hybrid enzyme.

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