PROPERTIES OF THE TRACE ENZYME IN HUMAN SERUM CHOLINESTERASE DEFICIENCY

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Summary. One-third of Eskimos with deficiency of serum cholinesterase had 2 to 8 percent of the enzyme activity of serum of normal persons, while the rest had no demonstrable cholinesterase. The properties of the purified trace enzyme resembled those of the normal enzyme so nearly that the trace enzyme is surely a cholinesterase and not a non-specific esterase. The differences were sufficient to show that the trace enzyme was not identical with the normal enzyme, but not great enough to determine whether or not a normal individual also has the trace enzyme.

The first description of a deficiency of serum cholinesterase reported a complete absence of enzyme activity (Liddell, Lehman and Silk, 1962), as have most subsequent reports. Goedde, Gehring and Hofmann (1965), however, studied two deficient patients in which 2 to 3 percent of the normal activity was present, but Hodgkin et al. (1965), using similar methods, found no evidence of activity in deficient serum.

Goedde and Altland (1968) and Gutsche, Scott and Wright (1967) showed by direct comparison that there were two types of deficient persons. One type has no cholinesterase activity whatsoever, while the second has 2 to 8 percent of the normal level of activity. An obvious explanation for the two types of deficiency is that they represent two different genetic defects. In one case, the defect results in an enzyme with low catalytic activity; in the other, the enzyme has no activity or is not produced at all.

In western Alaska about one percent of the population is deficient in serum cholinesterase, and 48 such persons are known. Thirty persons in 14 families are completely deficient and 15 persons in 12 families have a trace amount of enzyme. In one family a father has no activity while his son has the trace enzyme; the status of one person is unknown. That two genetic defects that are otherwise rare would occur in a population of about 5000 appears incredible and an alternate explanation was sought.

If it is assumed that the trace enzyme has a genetic locus distinct from the normal cholinesterase and that it is polymorphic in terms of presence or absence in the general population, the observed result can be explained. The polymorphism would be undetected in the normal person because the trace enzyme would be masked.

In this study, the trace enzyme was purified and compared with a purified normal enzyme to determine 1) if the trace enzyme is a cholinesterase or a non-specific esterase, and 2) if the properties of the trace and normal enzyme differed sufficiently so that the trace enzyme could be detected in the presence of the normal enzyme.

METHODS

Cholinesterase was determined by the method of Kalow and Genest (1957) in N/15 phosphate, pH 7.4, at 30°. Units of activity are micromoles of benzoylcholine hydrolyzed per minute under these conditions and specific activity is units per mg protein. Protein was determined spectrophotometrically (Warburg and Christian, 1942). Hydrolysis of benzoylcholine was measured by change of absorption at 240 mμ, of butyrylthiocholine at 230 mμ, and of α-naphthyl butyrate at 322 mμ.

Purification of normal enzyme - Plasma from outdated blood from a bank was tested for inhibition by dibucaine and fluoride. To 200 ml normal plasma (specific activity = 0.017) were added 40 g $(NH_4)_2SO_4$. The pH was adjusted to 3.5 with 2.5 M H_2SO_4 and the precipitate removed by centrifugation and discarded. The precipitate formed by an additional

15 g $(NH_4)_2SO_4$ per 100 ml solution was dissolved in a minimal volume of 0.1 M phosphate and dialyzed for 6 hours in 200 volumes of 0.01 M phosphate. All buffers used in these procedures were at pH 7.4 and contained 10^{-3}M EDTA and 0.1 percent 2-mercaptoethanol. The solution was passed through a 2.5 x 30 cm column of DEAE-cellulose which had been washed with 0.01 M phosphate. After the enzyme, more 0.01 phosphate was added, and the first colored fractions collected. Fractions with specific activity in excess of 0.35 were combined, and $(NH_4)_2SO_4$ fractionation repeated. The protein precipitating between 50 and 65 percent saturation was dissolved in a minimal volume of 0.01 M phosphate, and passed through a 2.5 x 30 cm column of Sephadex G-200 which had been washed with 0.01 M phosphate. The enzyme, which was eluted with 0.01 M phosphate, came through the column ahead of most of the protein. Fractions with specific activity greater than 14 were combined and to them was added an equal volume of $Ca_3(PO_4)_2$gel, (Tsuboi and Hudson, 1954), After centrifugation of the gel, the supernatant was discarded and 2 ml 0.05 M phosphate was added. The resulting eluate contained enzyme of specific activity 46 in 4 percent yield.

Purification of trace enzyme - To obtain a higher yield of trace enzyme a modified procedure was used. To 100 ml cholinesterase-deficient plasma (specific activity = 0.00043) was added 28 g $(NH_4)_2SO_4$ and the precipitate removed by centrifugation and discarded. The precipitate formed by adding a further 10.5 g $(NH_4)_2SO_4$ was dissolved in 4 ml M/15 phosphate. This solution was passed through a Sephadex G-200 column as above, and fractions with a specific activity greater than 0.004 combined. $(NH_4)_2SO_4$ (35 g per 100 ml solution) was added and the insoluble protein was removed by centrifugation and discarded. The addition of a further 14 g $(NH_4)_2SO_4$ per 100 ml resulted in an insoluble floating protein layer. The underlying liquid was removed and the protein dissolved in 2 ml M/15 phosphate. After dialysis for 6 hours against M/150 phosphate, the enzyme

solution was added to a DEAE-cellulose column (2 x 12 cm, washed with M/150 phosphate). The enzyme was eluted with 50 ml M/150 phosphate, 75 ml of this buffer containing 0.5 percent $(NH_4)_2SO_4$ and 75 ml containing 1.0 percent $(NH_4)_2SO_4$. Two ml samples were collected, and the most active of these gave an enzyme with a specific activity of 0.23 in 30 percent yield.

RESULTS

The properties of the normal and the trace enzymes that differed are shown in Table I. The only major differences were a greater heat stability and a lesser substrate inhibition of the trace enzyme. Other properties were so similar that only careful parallel measurements could distinguish the two. From inhibition constants, the dibucaine

TABLE I

Properties of the Normal Cholinesterase of Serum, and of the Trace Enzyme found in Deficiency

	Normal Enzyme	Trace Enzyme
Km, Benzoylcholine pH 7.4	10.1×10^{-6}	13.5×10^{-6}
pH 9.0	7.6×10^{-6}	12.5×10^{-6}
Optimum pH	9.0	8.8
Relative activity, α-Naphthylbutyrate/ Benzoylcholine	0.41	0.48
Ki, Dibucaine pH 7.4	4.9×10^{-7}	8.2×10^{-7}
pH 9.0	4.7×10^{-8}	6.8×10^{-8}
Ki, Procaine pH 7.4	6.1×10^{-6}	7.0×10^{-6}
Ki, Fluoride pH 7.4	1.6×10^{-5}	2.5×10^{-5}
$[I]_{\frac{1}{2}}$ Acetylcholine M	4.1×10^{-3}	4.4×10^{-3}
Activation by 10% ethanol, percent	63	45
Ki, Benzoylcholine	1×10^{-4}	50×10^{-4}
Inactivation at 58°, k (first order)	0.112	0.054

number (Kalow and Genest, 1957) of the normal and trace enzymes at 30° were calculated to be 77 and 72 respectively, while the corresponding fluoride numbers (Harris and Whittaker, 1961) at this temperature would be 34 and 30. Fluoride did not inhibit either enzyme at pH 9.0. The inhibition by acetylcholine and succinylcholine did not follow the expectation for a competitive substrate. In these cases the amount of inhibition is expressed as the concentration of inhibitor that lowered activity by 50 percent, $[I]_{\frac{1}{2}}$. The trace enzyme was relatively less active at lower pH, and more readily inactivated at higher alcohol concentrations.

The two enzymes were indistinguishable in inhibition by eserine (complete at 10^{-5} M), effect of temperature, inhibition by succinylcholine ($[I]_{\frac{1}{2}} = 6.0 \times 10^{-5}$ M), hydrolysis of butyrylthiocholine, and optimum ionic strength (0.09).

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