Preliminary Notes

The properties of neuraminidase-treated serum cholinesterase

The recent observation by SVENSMARK[1] that treatment of human serum with neuraminidase produced a decrease of the electrophoretic mobility of cholinesterase is particularly interesting in view of the fact that a crystalline serum mucoprotein was shown to have high cholinesterase activity[2] and purified preparations of this enzyme contain carbohydrate[3]. Since available preparations of serum cholinesterase are not homogeneous, the glycoprotein nature of this enzyme has not yet been settled. SVENSMARK's observation indicates, however, that human serum cholinesterase is an acid glycoprotein with several *N*-acetylneuraminic acid residues per molecule.

In the present investigation a purified cholinesterase preparation of human serum was treated with neuraminidase (a highly purified preparation of *Vibrio cholerae*) for 48 h at room temperature. After its isolation by preparative electrophoresis on a cellulose column, the treated enzyme was studied for substrate specificity and other properties, and compared with those of untreated cholinesterase. Fig. 1 demonstrates the electrophoretic separation of the untreated and neuraminidase-treated cholinesterase from a mixture of the two enzymes. The two components were first isolated separately by electrophoresis and the peak fractions mixed before re-electrophoresis. It can be noted that neuraminidase had no effect on the activity of this enzyme.

The properties of the two isolated esterases were compared by determining the substrate specificity, the Michaelis constants and the inhibitor constant for the esterase complex with prostigmine. The results obtained are illustrated in Table I, and indicate that the properties of the cholinesterase from which *N*-acetylneuraminic acid has been split off are identical with those of the original

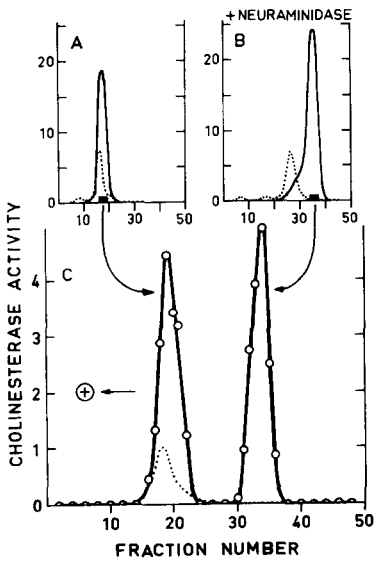

Fig. 1. Electrophoretic separation of a mixture of human serum cholinesterase and the same enzyme treated with neuraminidase. A solution of a purified enzyme preparation (Cohn fraction IV-6-4) was divided into two portions, A and B (corresponding to the two electropherograms A and B). Portion B was treated with neuraminidase (Behringwerke A.G., Germany) for 48 h at 23° and A was used as control. The two solutions were run separately on electrophoresis using cellulose columns[4]. The peak fractions (fractions used, marked on the two electropherograms) from each run were mixed and again run on electrophoresis (C). Cholinesterase activity (O—O) of the fractions was assayed with acetylcholine iodide as substrate[5] and expressed in μmoles of substrate split/h/0.2-ml fraction. Relative protein content (· · · · · ·) was measured by the Folin colour.

enzyme. Thus, *N*-acetylneuraminic acid, although it is a constituent of the natural enzyme, does not take part in the enzymic reaction.

TABLE I

PROPERTIES OF HUMAN SERUM CHOLINESTERASE TREATED WITH NEURAMINIDASE COMPARED WITH THOSE OF UNTREATED ENZYME

The enzymes used were isolated electrophoretically from a purified cholinesterase preparation (Cohn fraction IV-6-4). pK_m was determined with acetylcholine iodide as substrate (Warburg technique).

	Untreated enzyme	*Enzyme treated with neuraminidase*
Hydrolysis rates (b_{30})		
acetylcholine	59	68
propionylcholine	124.5	122.5
butyrylcholine	162	165.5
pK_m	2.55	2.51
pK_i (prostigmine bromide)	7.11	7.06

The fact that the electrophoretic mobility of the "new" cholinesterase is much lower than that of the globulins with which it normally runs in electrophoresis and from which natural cholinesterase is difficult to separate makes it possible to prepare a serum cholinesterase preparation of high purity. Actually, the specific activity (10000–20000) of the peak fractions (Fig. 1) of the isolated, neuraminidase-treated enzyme obtained in the present investigation is one of the highest value reported so far for this enzyme[5].

Cholinesterase with abnormal low electrophoretic mobility may occur naturally when neuraminidase is present in the blood. This explains, in such cases at least, the appearance of cholinesterase activity in two fractions on electrophoresis, reported by some authors during the last years[6].

Cholinesterase from horse serum behaves similarly to that of human serum with respect to the effect of neuraminidase. Neither arylesterase (human, horse) nor aliesterase (horse) of blood serum change their electrophoretic mobility after treatment with neuraminidase. These enzymes, therefore, probably contain no *N*-acetylneuraminic acid.

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