Treatment of Horse Serum Cholinesterase with Sialidase

EDITH HEILBRONN

Research Institute of National Defence, Sundbyberg 4, Sweden

The effect of bacterial sialidase on the electrophoretic properties of human serum cholinesterase suggests that this enzyme is a sialoprotein [1]. A sample of 2 200 fold purified cholinesterase from horse serum, prepared in this laboratory and analyzed for its amount of sialic acid, revealed 3.2 % of this acid [2]. This preparation, however, still does not represent the pure enzyme. It is therefore possible that the sialic acid belonged to other proteins. Further experiments were carried out (on a preparation with a lower degree of purity) in order to answer the question.

Experimental. 25 mg of a preparation obtained by ammonium sulfate fractionation [3] (purification factor about 200) were dissolved in 10 ml of 0.05 M acetate buffer pH 5.5. To the same solution 2 mg of sialidase (Behringwerke, Germany) were added. The solutions were kept at 25°C for 30 days. Twice a week the enzyme activity was determined with an automatic recording titrator at pH 8.0 and 25°C with 5×10^{-3} M butyrylcholine iodide as substrate. The mobility of the enzyme was controlled with paper electrophoresis in Tris buffer pH 8.9 (Schleicher and Schüll paper No. 2043B, 40 × 410 mm, 200 V). The protein was stained with amidoschwarz and the cholinesterase activity was demonstrated with indoxylacetate [2]. Sialidase alone did not stain at the concentration used.

Results. The paper electropherograms of the cholinesterase preparation showed three protein bands. After treatment with sialidase only one band was seen already after one day. The position of the enzyme was changed and closer to the starting line (see Fig. 1). Zone electrophoresis on cellulose of the sialidase treated cholinesterase preparation revealed only one protein peak. Without sialidase treatment this preparation is resolved into three or four peaks [2]. The activity of the sialidase treated cholinesterase solution was constant during 30 days. The results indicate that horse serum cholinesterase as well as some of the impurities of the used preparations are sialoproteins.

1. Svensmark, O. *Acta Physiol. Scand.* **52** (1961) 267.
2. Heilbronn, E. *Biochim. et Biophys. Acta* (1962). *In press.*

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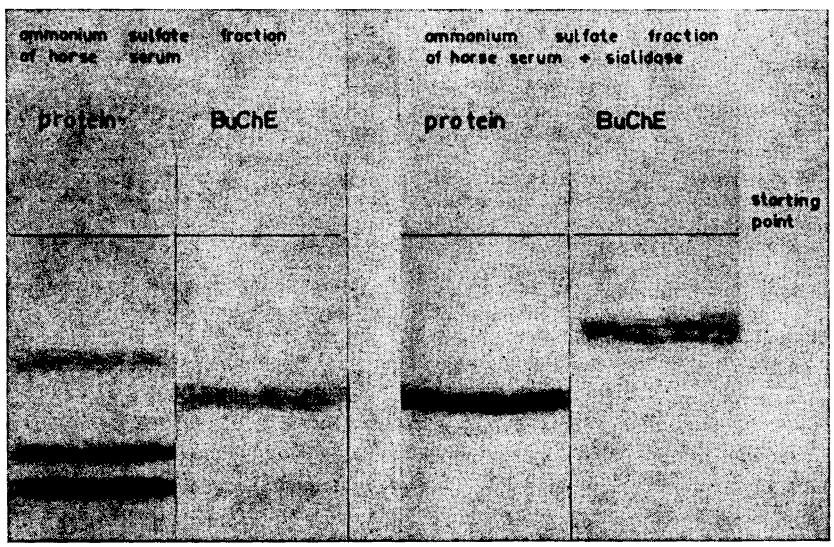

Fig. 1. Paper electropherograms of a cholinesterase preparation from horse serum before and after treatment with sialidase.