Alteration in the Electrophoretic Mobility of Alkaline Phosphatases after Treatment with Neuraminidase

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Attempts to distinguish between the alkaline phosphatases (orthophosphoric monoester phosphohydrolases, EC 3.1.3.1) obtained from different human tissues have been based on variations in kinetic properties, reactions with certain inhibitors, and differences in immunological, chromatographic and electrophoretic behaviour. Of these criteria, differences in mobility on starch-gel electrophoresis have received considerable attention recently, and essentially comparable results of investigations of this type have been reported from several Laboratories (Wilkinson, 1965). The existence of electrophoretically distinct alkaline-phosphatase components in human blood serum has also been noted. In the absence of highly purified preparations of human alkaline phosphatase it has not been possible to decide whether these electrophoretic differences represent fundamental variations in molecular architecture between the enzymes from different sources, or whether they indicate differences that do not affect the primary structure of the enzyme molecule, e.g. due to interaction of the enzyme protein with non-enzymic entities.

A decrease in electrophoretic mobility after treatment of certain human blood serum proteins with neuraminidase (EC 3.2.1.18) has been demonstrated by Schultze (1962), and Robinson & Pierce (1964) showed that, of two alkaline-phosphatase components in human serum, one was retarded by neuraminidase treatment. The action of neuraminidase also converts the heterogeneous pattern seen on starch-gel electrophoresis of human-kidney alkaline phosphatase into a single active enzyme zone (Butterworth & Moss, 1966). The present report describes the effect of neuraminidase on the electrophoretic properties of human-liver and -intestinal alkaline phosphatase, both in unfractionated tissue extracts and after several purification stages.

Phosphatase-containing extracts of fresh post-mortem specimens of human liver and small intestine were made by Morton's (1950) butanol method. Further purification of the tissue extracts was achieved by gel filtration on a column of Sephadex G-150 (Pharmacia A.B., Uppsala, Sweden) followed by column chromatography on DEAE-cellulose with gradient elution at pH 7.7 (Grossberg, Harris

& Schlamowitz, 1961). Tissue extracts or chromatographic fractions (0.1 ml.) were incubated with 0.4 ml. of neuraminidase solution (from *Clostridium perfringens*; Sigma Chemical Co. Ltd., type V: 1 mg./ml. in tris buffer, pH 7.7) for 24 hr. at 37°. Treated and untreated alkaline-phosphatase solutions were submitted to horizontal starch-gel electrophoresis (Smithies, 1955) in a discontinuous tris–citrate–borate buffer system at pH 8.6 (Poulik, 1957) for 17 hr. at an initial voltage of 6 v/cm. After electrophoresis, alkaline-phosphatase zones were located on the gel as described by Estborn (1959).

The electrophoretic mobility of the major alkaline-phosphatase zone of liver extract was considerably decreased by neuraminidase action, and a minor enzyme zone close to the origin was also retarded (Fig. 1a). Neuraminidase treatment had little effect on the electrophoretic pattern of intestinal alkaline phosphatase, only a small amount of activity being detected in the region corresponding to the main retarded zone of liver phosphatase (Fig. 1b). Similar effects were observed when the unfractionated tissue extracts were replaced by enzyme fractions partially purified by gel filtration and anion-exchange chromatography (Figs. 1c and 1d). The relative lack of effect of neuraminidase treatment on intestinal phosphatase was not due to incomplete action of neuraminidase as a result of inactivation; at the end of the 24 hr. incubation period the neuraminidase was still active, as was shown by adding liver extract and continuing the incubation, when the retarding effect of neuraminidase on liver phosphatase was still produced. This experiment also demonstrated that the lack of activity on intestinal extract was not due to the presence in the extract of factors that inhibited the neuraminidase action. In all experiments, the total alkaline-phosphatase activity was unaffected by neuraminidase treatment when compared with similarly incubated controls. Incubation with lysozyme and hyaluronidase, enzymes of related specificity, produced no effect on electrophoretic mobility.

The differential effect of neuraminidase on liver and intestinal alkaline phosphatases provides a further distinction between the latter phosphatase and those from other tissues. The effect does not
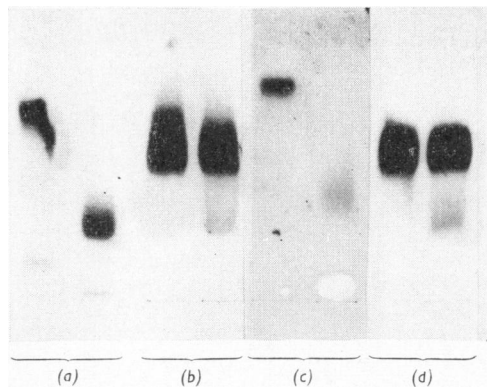
Fig. 1. Comparison of alkaline-phosphatase zones of human liver and small intestine on starch-gel electrophoresis at pH 8·6, showing the effect of incubation with neuraminidase. (a) Liver extract; (b) small-intestinal extract; (c) liver alkaline phosphatase after fractionation by gel filtration and anion-exchange chromatography; (d) intestinal alkaline phosphatase after fractionation by gel filtration and anion-exchange chromatography. In each case, the sample incubated with neuraminidase is on the right and the control incubated without neuraminidase on the left. The sample slots are near the bottom and the anode at the top.

aminidase on intestinal alkaline phosphatase may be due to the presence of a second, sialic acid-containing, phosphatase; it was, however, seen when chromatographic fractions of intestinal phosphatase were used (Fig. 1d), as well as with unfractioned tissue extracts, suggesting that fractionation had failed to remove any such second component.

The slow-moving phosphatase zone near the origin in liver extracts has been attributed to formation of a complex of the phosphatase with lipoprotein (Moss, 1962). The retardation of this zone by neuraminidase treatment supports this suggestion, since removal of sialic acid residues would be expected to affect the electrophoretic mobility of such complexes as well as that of the free enzyme molecules, provided that the sialic acid residues were not rendered inaccessible to neuraminidase action by the structure of the complex.

The present findings support the implication of other studies that intestinal alkaline phosphatase differs from phosphatases from other tissues, and they indicate a possible basis for the different electrophoretic mobilities of these enzymes.

seem to be due to impurities in the enzyme preparations, since it persists after several purification stages. These purification procedures remove low-molecular-weight substances and certain sialic acid-containing proteins such as orosomucoid and haptoglobin, which, by interacting with alkaline phosphatase, might give rise to the effect seen on treatment with neuraminidase. The present findings agree with those of Robinson & Pierce (1964) that a phosphatase zone of human serum, which was presumed to arise from intestine since it was inhibited by L-phenylalanine (Fishman, Green & Inglis, 1962), was not retarded on starch-gel electrophoresis after neuraminidase treatment, and with the observation of Engström (1961), who detected no sialic acid in purified calf-intestinal alkaline phosphatase. The small effect of neur-

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