COMMUNICATIONS

Differential Assay of Human Hexosaminidases A and B

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Interest in the two forms (A and B) of *N*-acetyl- β -glucosaminidase first described by Robinson & Stirling (1968) has increased as a result of observations by Sandhoff (1969) and Okada & O'Brien (1969) that the A form is absent in Tay-Sachs disease and by Dance, Price, Cattell, Lansdell & Richards (1970) that the appearance of the B form in urine is a sensitive indicator of kidney damage. A rapid and reliable method for determining relative activities of the two forms is required, and we have compared several methods based on differences in their physical properties.

Comparison of the isoenzymes by starch-gel electrophoresis can be made quantitative by using 4-methylumbelliferyl *N*-acetyl- β -glucosaminide as the substrate and scanning the pattern of fluorescent zones. Excessive substrate hydrolysis must be avoided.

On selective denaturation by gentle heat the A form is destroyed most rapidly, and the denaturation curves of tissue homogenates are biphasic at pH 5.5 at 50°C. Extrapolation of the second gradient to zero time gives an estimate of the form B present, or assays based on denaturation for the precise half-life of form A can be used, assuming that form B is unaffected. These methods may be subject to gross error, since the A form is apparently converted in the process into the more stable B form. In our original study on the human kidney enzyme (Dance, Price, Robinson & Stirling, 1969) we assayed the two forms after separation on DEAE-cellulose columns with an NaCl eluent at pH 6.0. A simplified version of this procedure is rapid, effective and useful for small volumes of tissue homogenates or pathological urine. The sample (typically 1 ml of fivefold-diluted urine or 0.5% tissue homogenate) is mixed with 1 ml of a 10% suspension of Whatman DE32 ion-exchange cellulose, pre-equilibrated in 10 mM-phosphate buffer, pH 6.0. The suspension is continuously inverted for 15 min and then centrifuged. The activity of the supernatant (B form) is compared with that of the original sample (A and B). To check the recovery of activity the A form adhering to the cellulose can be displaced in 0.2 M-NaCl.

In our hands this last method is the easiest and

most reproducible, it requires the smallest amount of sample and can be carried out in the shortest time.

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The Lipases Produced by *Anaerovibrio lipolytica* in Continuous Culture

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Earlier publications (Hobson & Summers, 1966; Henderson, Hobson & Summers, 1968) have described the phenomenon, in continuous cultures of the rumen bacterium *Anaerovibrio lipolytica*, of two growth rates at which maximal amounts of lipase activity can be detected in the cell-free culture medium. As part of an attempt to explain this phenomenon, samples of enzyme produced at each of these growth rates have been purified and their properties compared. The purification process involved precipitation and adsorption of the enzyme from large volumes of medium by using ammonium sulphate and cellulose powder. The enzyme was eluted from the cellulose powder with phosphate buffer and after dialysis and ultrafiltration further purified on columns of Sephadex G-100 and Sephadex G-200. The enzymes collected at each of the growth rates studied were excluded from Sephadex G-200. Electrophoresis of these purified samples showed the presence of more than one component in each enzyme preparation.

The characteristics compared in studies of lipases purified on Sephadex G-200 were: effect of pH on activity, effect of temperature on activity and the rates of hydrolysis of different triglyceride substrates.

The pH at which optimum activity was obtained was 7.4 in both cases, with only a small difference in the shapes of the curves obtained when enzyme activity was plotted against incubation pH.

The two enzyme preparations differed in the temperature at which maximum hydrolysis of

substrate occurred. The enzyme produced at the lower growth rate had optimum activity at 20–22°C, whereas the enzyme from the higher growth rate showed maximum activity at 26°C.

There were notable differences when the activities of the enzymes were assayed with a range of different triglycerides as substrates. The enzyme produced at the lower growth rate showed most rapid hydrolysis of trilaurin, whereas trioctanoin was most rapidly hydrolysed by fresh preparations of enzyme from the higher growth rate. However, during storage for 30 days at –20°C this latter preparation lost 50% of its activity towards trioctanoin but the activity towards trilaurin was unchanged. This change towards the first enzyme may indicate an alteration to a more stable configuration or perhaps the preferential deactivation of one enzyme in a multiple enzyme system.

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Adenylate Deaminase Activity in a Number of Species

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The activities of AMP deaminase and adenosine deaminase in the absence and presence of ATP and GTP were investigated in the lung, liver, spleen, intestine, heart and skeletal muscle of the mouse, rat and guinea pig in an examination of the pathway of AMP catabolism in these tissues.

The activity of adenosine deaminase was not affected by ATP or GTP in any of the tissues studied. The activity of AMP deaminase was increased in the presence of ATP, but GTP had little effect on the activity of this enzyme except in skeletal muscle, where an activation similar to that of ATP was produced.

The effect of GTP on AMP deaminase was studied in more detail in rabbit skeletal muscle, where the activity of the enzyme is high and a simple purification method is available (Smiley, Berry & Suelter, 1967). The properties of AMP deaminase in the homogenate and after purification were shown to differ both in the effect of substrate concentration and of GTP on activity. Evidence is presented that these differences may be due to an alteration in the physical state of the enzyme on purification.

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Purification of the Low-Molecular-Weight Form of Adenosine Deaminase from Rabbit Intestine

By C. O. PIGGOTT and T. G. BRADY. (*Department of Biochemistry, University College, Cork, Irish Republic*)

During a survey of the molecular weights of adenosine deaminases from mammalian sources by Murphy, Brady & Boggust (1969) it was found that in the rabbit duodenum two molecular forms of the enzyme existed with molecular weights of 260 000 and 34 000 respectively. It was therefore decided to study the enzymes in this tissue with a view to characterizing these forms. The present communication outlines a scheme for the purification of the low-molecular-weight form (mol.wt. 34 000) of the enzyme.

The procedure involves initial extraction with acetate buffer, pH 5.5, precipitation and fractional re-extraction with $(\text{NH}_4)_2\text{SO}_4$ (Ghosh & Fishman, 1968), chromatography on DEAE-cellulose, pH 5.8, and final purification on Sephadex G-75 (superfine grade). The recovery varied between 20 and 30% and the final specific activity of the preparation was 300 units/mg. The enzyme was shown to be both chromatographically and electrophoretically pure.

Throughout the purification the ratio of the two forms of the enzyme was monitored by thin-layer gel filtration (Morris, 1964). The chromatograms were developed by covering the plate with a solution of agar containing adenosine and Phenol Violet, and measured quantitatively by reflectance densitometry. DEAE-cellulose chromatography resulted in a partial separation of the two forms, and Sephadex chromatography completed the separation. The thin-layer gel-filtration technique also proved to be of great analytical use in determining the ratio of the molecular forms in individual rabbit intestines.

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