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Isoenzymes of N-Acetyl- β -Glucosaminidase from Human Liver and Serum: Separation by Electrofocusing in Thin Layers of Polyacrylamide Gel

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Summary: Isoenzymes of N-acetyl- β -glucosaminidase can be separated by electrofocusing in thin layers of polyacrylamide gel. Patterns of isoenzymes from human liver tissue and serum show changes in liver diseases. Preliminary results indicate, that N-acetyl- β -glucosaminidase isoenzyme A is increased in liver tissue from patients with chronic liver diseases in relation to the activity of mesenchyme reaction. The described method will be discussed in comparison with known separation techniques.

Isoenzyme der N-Acetyl- β -glucosaminidase aus Lebergewebe und Serum des Menschen: Trennung durch Elektrofokussierung in Polyacrylamidgel-Platten

Zusammenfassung: Isoenzyme der N-Acetyl- β -glucosaminidase werden durch Elektrofokussierung in Polyacrylamidgel-Platten dargestellt. Isoenzymmuster aus Lebergewebe und Serum zeigen bei Lebererkrankungen Veränderungen. Erste Ergebnisse weisen darauf hin, daß das Isoenzym A der Glucosaminidase entsprechend der Aktivität der Mesenchymreaktion im Lebergewebe bei chronischen Lebererkrankungen erhöht ist. Die beschriebene Methode wird mit den bisher bekannten Darstellungsverfahren der N-Acetyl- β -Glucosaminidase-Isoenzyme verglichen.

Introduction

N-Acetyl- β -glucosaminidase, a lysosomal glycosidase (glucosaminidase, β -hexosaminidase, EC 3.2.1.30) is involved in the breakdown of glycosaminoglycans from connective tissue (1–5). As described previously, several forms of glucosaminidase can be separated by gel electrophoresis (6), ion exchange chromatography (6) and analytical electrofocusing in polyacrylamide gel (7).

The demonstration of isoenzymes in tissue from patients with gangliosidosis has been used clinically (5, 8, 9). Changes of the isoenzyme patterns of glucosaminidase in serum during pregnancy (7, 10, 11) and in urine from patients with renal disease (12) are well known.

In this report we demonstrate a method for the separation of glucosaminidase isoenzymes and the preliminary results of their clinical application. This separation method by electrofocusing in thin layers of polyacrylamide gel is easy to handle and therefore presents the opportunity for a wide range of clinical uses.

Material and Methods

Thin layers of polyacrylamide gel (50 g/l, 10 g/l ampholytes) and an electrofocusing system (Multiphor) were purchased from LKB München, Bundesrepublik Deutschland, 2-naphthyl- β -N-acetyl-glucosaminide and *p*-nitro-phenyl- β -N-acetyl-glucosaminide from Koch-Light-Lab., Colnbrook, U.K., *p*-nitro-benzolazo-2,5-dimethoxy-aniline (fast black) from Serva, Heidelberg, Bundesrepublik Deutschland. All other chemicals of analytical purity were purchased from Merck, Darmstadt, Bundesrepublik Deutschland.

Whole blood was allowed to clot at room temperature (60 min) and serum was obtained after centrifugation at 3000 g for 10 min. Liver tissue (20 g/dl) was homogenized 3 $\times$ 1 min in 0.15 mol/l NaCl, containing 0.001 mol/l EDTA with an Ultraturrax, supplied by Janke and Kunkel, Stauffen, Bundesrepublik Deutschland. After twice freezing and thawing, the 3000 g supernatant and serum were examined for both enzyme activity (2, 13) and protein (14).

After dialysis against distilled water for 3 hours, the isoenzymes were separated by electrofocusing in thin layers of polyacrylamide gel. 60 μ l serum (5.2 mg protein) and 20 μ l supernatant (60 μ g protein) were applied to a filter paper (5 $\times$ 10 mm) on the gel surface between the electrodes with 20 mm distance from the cathode. Current and time were chosen as recommended by the producer.

All described operations were carried out at + 4°C, unless otherwise indicated; the electrofocusing procedure was run at + 10°C by means of a cooling plate. Isoenzyme bands were stained (15) with 2-naphthyl- β -N-acetyl-glucosaminide and *p*-nitrobenzoyl-2,5-dimethoxyaniline (fast black). Heat inactivations were carried out according to *O'Brien* (16). Incubations with a detergent to dissolve suspected reversible enzyme-protein linkage were performed with Triton X 100 (final concentration 0.5 g/l) for 30 min at 20°C. The pH-gradient was determined directly by a surface pH-electrode (e. g. Type LOT 403-30-M8, Ingold, Zürich, Switzerland).

Densitograms of stained gels and estimation of areas of the densitograms, corresponding to different isoenzyme bands, were carried out by a Vitatron densitometer. Activity of different glucosaminidase isoenzymes are given in % of measured areas of all enzyme bands in the densitogram (= 100%).

According to previous results and criteria (17), the activity of the mesenchyme reaction was determined by histological examination of the liver tissue (+ to +++). Criteria are: Degree of portal, intralobular fibrosis with collagen accumulation. Amount of mesenchyme cells, i. e. fibroblasts and macrophages in portal tracts and in the parenchyma of the liver.

Results and Discussion

Homogenates from human liver submitted to electrofocusing in thin layers of polyacrylamide gel showed two groups of isoenzyme bands (fig. 1), one which migrated nearer to the anode with a pH at 5.0 at the maximum of enzyme activity, the other one with a pH at 7.3. These two isoenzymes were those of glucosaminidase A (pH 5.0) and B (pH 7.3), according to *Sandhoff* (9). Between pH 5.5 and 7.0 a few enzyme bands with less activity are visible, which are designated as P isoenzymes (10, 11).

Fig. 1. Isoenzymes of glucosaminidase from liver tissue, separated by gel electrofocusing. Left side: normal liver (40 U/g protein), right side: cirrhotic liver (72 U/g protein).

Patterns of isoenzymes from normal and diseased human livers are shown in figure 1 and figure 2. The relation between glucosaminidase isoenzyme A and activity of the mesenchyme reactions in 9 histologically examined specimens of liver tissue is shown in figure 2. These first results, controlled by another group of 16 specimens

Fig. 2. Isoenzyme screening of glucosaminidase and activity of mesenchyme reaction (+ to +++). Relation between isoenzyme A and B + P was determined by comparing the areas (total = 100%) under the densitograms of the stained isoenzyme bands.

- 1.-3. Normal liver
4. Highly active biliary cirrhosis of the liver
5. Highly active alcoholic hepatitis
6. Active alcoholic cirrhosis of the liver
7. Active alcoholic hepatitis
8. Active cirrhosis of the liver, caused by viral hepatitis
9. Alcoholic cirrhosis of the liver, little activity.

from normal and diseased livers, indicate that isoenzyme A has increased according to the activity of the mesenchyme reaction in chronic liver diseases. Previous investigations (17) showed an increase of whole glucosaminidase activity in liver tissue and serum from patients with chronic liver diseases, related to the activity of the mesenchyme reaction.

In this study, we reported that the catalytic concentration of glucosaminidase was 17 ± 0.28 U/l serum

($x \pm \text{SEM}$) in healthy persons. In patients with chronic liver diseases the catalytic concentration increases up to 45 ± 3.7 U/l (mesenchyme activity +++), 28.5 ± 1.1 U/l (++) and 22.8 ± 1.0 U/l (+). Activity of glucosaminidase in liver tissue, taken by needle biopsy, was increased 2–3 fold in chronic liver diseases. These results indicate that elevation of glucosaminidase is caused in part by elevation of glucosaminidase isoenzyme A.

Bands of isoenzymes from a cirrhotic liver before and after heat inactivation are illustrated in figure 3. As reported by O'Brien (16), the activity of the isoenzyme A disappeared after heat treatment.

The glucosaminidase activity of serum is less than that of tissue extracts. Therefore normal serum shows only faint bands in the areas corresponding to the A (one band), P (several bands), and B (one band) forms of the liver. Serum glucosaminidase from patients with acute hepatitis shows an increase of A and P isoenzymes (fig. 4, right side), while serum from patients with cirrhosis of the liver shows an increase of isoenzyme A (fig. 4, left side), according to the isoenzyme patterns of cirrhotic livers.

Between A and P forms of glucosaminidase from serum a group of isoenzymes has been found, whose identity is not clear (7, 12). Heat treatment of serum glucosaminidase caused a decrease of these isoenzymes and of isoenzyme A.

After incubation with Triton X 100 a change of the isoenzyme patterns both from liver tissue and serum did not occur. Therefore, it is improbable, that the described isoenzyme bands are caused by reversible enzyme-protein complexes.

Fig. 3. Patterns of isoenzymes from cirrhotic liver before (right side) and after heat treatment (left side).

Fig. 4. Isoenzymes of glucosaminidase from serum, separated by gel electrofocusing. Right side: acute hepatitis, left side: liver cirrhosis. Whole enzyme activity of glucosaminidase (catalytic concentration: U/l serum) from left to right side: 49.7, 22.8, 42.1, 34.5.

The described method for the separation of isoenzymes of glucosaminidase offers the following advantages in comparison with the known separation techniques:

1. Compared to electrophoresis (6, 12) and ion exchange chromatography (6) a direct separation is possible without expensive pretreatment.
2. In comparison with other electrofocusing techniques the more efficient cooling of the gel offers a considerable shortening of the experimental time. Therefore the decrease of enzyme activity during electrofocusing is less.
3. Compared to gel electrofocusing in tubes many samples can be run simultaneously under identical conditions. Machine-made and controlled fabrication of the thin layer gel gives identical conditions from charge to charge.

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