DEMONSTRATION OF THE MULTIPLICITY OF N-ACETYL-BETA-GLUCOSAMINIDASE BY ELECTROPHORESIS

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Summary

The present report describes the results of electrophoretic study of the enzyme, N-acetyl-β-glucosaminidase, which is involved in the metabolism of glycoproteins and glycolipids, and widely distributed in living organisms.

In this study of the enzyme, bovine liver, spleen, and kidney were homogenized and centrifuged, and the supernatant was used. By the method of Ornstein-Davis, polyacrylamide gel disc electrophoresis was carried out. When electrophoresis was completed and the gels were taken out and incubated, a few bands were observed on the gels. By the densitometric study following the electrophoretic study the peaks corresponding to the bands on the gels were recognized.

It is known that N-acetyl-β-glucosaminidase is involved in the metabolism of glycoproteins and glycolipids and widely distributed in living organisms. Also, relatively high activity of this enzyme has been detected in such organs as liver, spleen, kidney and endocrine organs, etc. Up to this time, the enzyme activity has been demonstrated by using samples of serum, synovial fluid, and other organ materials. The activities of the enzyme have also been determined in the samples of the serum from gravid women and patients with liver and kidney disorders or diabetes mellitus (1-10).

The present report describes the results of electrophoretic study of the enzyme.

Material and Methods

Bovine liver, spleen, and kidney were collected post-mortem and stored in a deep-freezer. Each sample was well washed with physiological saline solution to remove fat tissue of it as much as possible. 4 ml. of ice-cold physiological saline solution was added to each 1 gm. of the sample, and the samples

were homogenized by using loosely-fitting teflon pestle in a Potter-Elvehjem type motor-driven tissue homogenizer. The suspension of each sample was centrifuged at about 10,000 r.p.m. for 10 to 20 minutes at 0°C in a high speed centrifuge. The sediment was discarded, and the supernatant was stored in a deep-freezer, to be kept till it was required.

By a modification of the method of Ornstein-Davis (11, 12), disc electrophoresis using 4.5 to 5% polyacrylamide gel was performed.

In each 0.5 x 9 to 10 cm. glass column, 0.8 to 1 ml. of lower gel was polymerized with ammonium persulfate and large-pore gel containing 5 to 50 μl. of the sample was then photopolymerized.

A small amount of bromophenol blue was added as a tracking dye, and Tris-glycine buffer pH 8.3 was carefully layered over the gels. The columns were run simultaneously at a low temperature in the Tris-glycine buffer under the current of 1.5 to 3 mA per column for a few hours, at which time the tracking dye reached near the bottom of the column. The gels were then removed from the columns and after being immersed with 0.1 M citrate buffer pH 4.1, they were either incubated in 20 to 100 ml. of the 0.1 M citrate buffer with 3 to 30 mg. of naphthol AS-BI N-acetyl-β-glycosaminide (naphthol AS-BI: 7-bromo-3-hydroxy-2-naphth-o-anisidide) and 10 to 50 mg. of Fast Red Violet LB salt (Sigma Chemical Co.) at 37°C for 30 minutes, or left overnight at room temperature.

Results and Discussion

The results are shown in Fig. 1. The enzyme activity showed a red violet tint band in the gel against the light yellow background.

With the bovine liver extracts (BL), two bands which exhibited different mobilities, fast moving and slow moving, were observed. Both bands of these mobilities migrated to the anode side. In the case of kidney (BK), the band located at the anode side of the gel, which exhibited a fast moving band mobility, was a little narrower than those obtained with liver and spleen (BS).

The content of these two components differed from one organ to another, as shown by the intensity of staining.

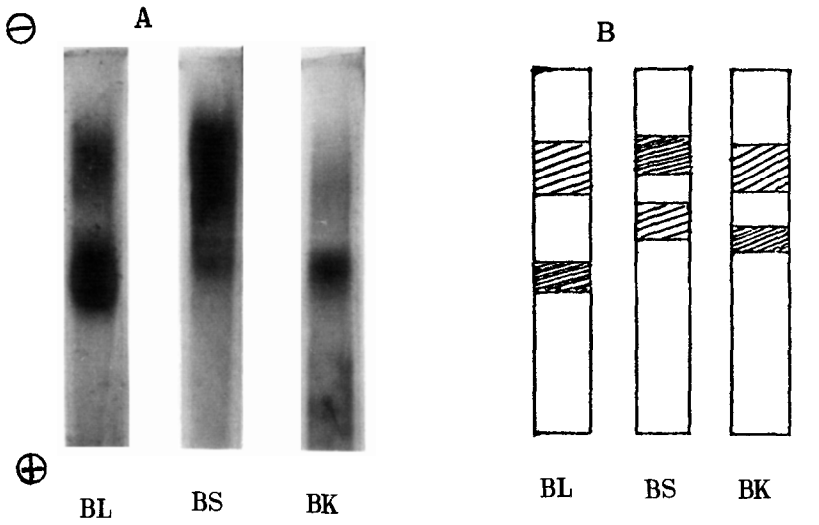

Fig. 1.
A: the gel electrophoresis pattern of the enzyme derived from the homogenate of bovine organs: liver, spleen, and kidney. B: schmatic representation of A. From left to right ; BL : liver, BS : spleen, BK : kidney. The upper end of the gels (on cathode side) was laid with samples. The lower end lies on the anode side. The electrophoresis was carried out at a low temperature in Tris-glycine buffer under the current of 1.5 to 3mA per column with the cathode in the upper chamber for a few hours. After that the gels were taken out and incubated with substrate and dye.

In liver sample, densitometric study of the gels obtained through the above procedures disclosed two peaks respectively corresponding to the mobility of the bands, while in kidney and spleen samples each one peak exhibited a pattern of peak different from the liver's (Fig. 2.).

It is now accepted that the enzyme is a lysosomal one involved in the metabolism of glycoproteins and glycolipids, and its physiological significance including its nature can be found in many articles (1-10, 13, 14). The present study was performed by means of electrophoresis and by referring to Hayashi's histochemical method (15) and it resulted in the demonstration of the enzyme pattern with a few fractions.

Electrophoresis was also performed on the acidic buffer (16). And it

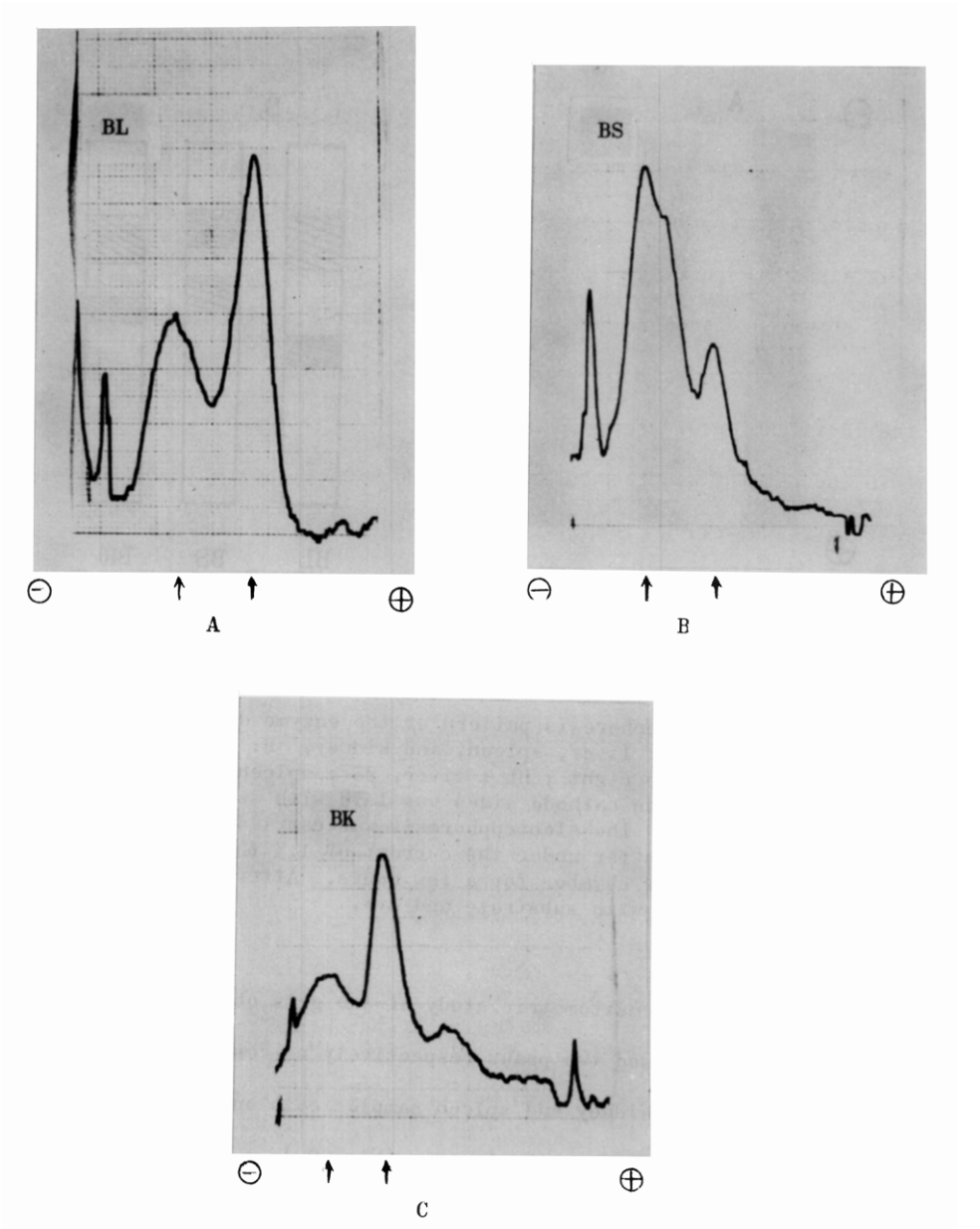

Fig. 2.
Densitometric tracing of the electrophoresis pattern of the enzyme of Fig. 1 derived from the homogenate of the bovine organs, measured by means of a filter at 520 mμ . A : liver (BL), B : spleen (BS), C : kidney (BK). On each of the illustrations A, B, C, the origin (on cathode side) lies on the left side, and arrows indicate the major peaks corresponding to the components; the peaks of densitometric curve were found well corresponding to the bands of the gels.

seemed that better results were brought about on the alkaline condition than on the acidic condition except for the case of kidney which presented narrower and sharper bands on the acidic condition than on the alkaline condition.

In staining the gel, Fast Red Violet LB Salt was employed, and in the gel the localization of the enzyme activity was developed in a distinct red violet showing a sharp contrast to the yellow background.

As observed, the enzyme from the liver material presented two bands and two peaks which were well corresponding to one another, respectively in regard to the localization of staining and densitometric trace.

In the case of other organs, spleen and kidney, peaks of densitometric curve were found well corresponding to the bands of the gels, too.

The results thus obtained are suggestive of the presence of at least two kinds of molecular forms of the enzyme in bovine organs including liver.

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