Note

 β -N-Acetylhexosaminidase Obtained from Chicken Liver and Chicken Serum

Yuji OGAWA and Ryo NAKAMURA

Department of Food Science and Technology,
Nagoya University, Chikusa-ku,
Nagoya 464, Japan

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β -N-Acetylhexosaminidase is a typical lysosomal enzyme and has been shown to be distributed in a wide variety of living tissues. In a previous work,¹⁾ we purified this enzyme from both egg white and lysosomal fraction of hen oviduct, and showed that the subunit molecular weight of the egg white enzyme is larger than that of the oviduct enzyme. This result suggests that the subunit molecular weight of extracellular NAHase is larger than that of intracellular NAHase. To investigate this possibility, NAHase was separated from other living tissues which have an extracellular enzyme (serum) and an intracellular enzyme (liver), and their subunit molecular weights were compared.

Both the liver and serum were separated from freshly slaughtered White Leghorn hen. The liver was rinsed in ice cold 0.85% saline, cut into small pieces and homogenized in ice cold 20 mM phosphate buffer (pH 7.0) containing 0.25% sucrose and 0.2 M KCl. The homogenate was centrifuged at $500 \times g$ for 5 min. The resulting supernatant was centrifuged at $18000 \times g$ for 30 min, resulting in the lysosomal fraction as a pellet. NAHase was solubilized from this lysosomal fraction by a three-fold freeze-thawing process. Both the liver and serum NAHase were partially purified by immunoaffinity chromatography as described in the previous work.¹⁾ NAHases obtained from hen oviduct and egg white in the previous work were also used for comparison.

SDS-polyacrylamide gel electrophoresis (12.5% acrylamide gel, SDS-PAGE) was performed by the method of Laemmli.²⁾ After electrophoresis, the proteins were transferred to nitrocellulose sheets using the electrophoretic blotting procedure of Towbin *et al.*³⁾ NAHases on the nitrocellulose sheet were incubated with anti-NAHase IgG (rabbit), washed with saline (about five times during 30 min) and incubated with the fluorescein isothiocyanate (FITC)-conjugated goat anti rabbit IgG. After incubating for 1 hr, the sheet was washed with saline and photographed under long-wave (365 nm) UV light through a yellow filter. The molecular weight of the protein band was determined using the following standard protein mol-

ecules; phosphorylase b (9.4×10^4), bovine serum albumin (6.7×10^4), ovalbumin (4.3×10^4), carbonic anhydrase (3.0×10^4) and trypsin inhibitor (2.01×10^4). An SDS-polyacrylamide gel electrophoretogram of these standard proteins was electrophoretically blotted and stained with Amide Black 10B.

Figure 1 shows the double immunodiffusion of four NAHases obtained from different sources, namely, liver, oviduct, serum and egg white, against anti NAHase IgG obtained in the previous work.¹⁾ In this figure, the enzyme precipitin lines fused with one another and no spur was

FIG. 1. Ouchterlony Double Immunodiffusion of Four NAHases.

1, oviduct lysosomal NAHase; 2, liver lysosomal NAHase; 3, egg white NAHase; 4, serum NAHase; AB, anti-NAHase IgG.

FIG. 2. Immunological Detection of Four NAHases in the SDS-PAGE Patterns.

1, oviduct lysosomal NAHase; 2, liver lysosomal NAHase; 3, egg white NAHase; 4, serum NAHase.

detected, indicating that the four enzymes may well have the same antigen determinants.

Figure 2 shows SDS-PAGE patterns of the four NAHases illustrated in Fig. 1. In Fig. 2, it can be clearly seen that the subunit molecular weight of liver NAHase is the same as that of oviduct NAHase (MW=53000), and that of serum NAHase is the same as that of egg white NAHase (MW=68000).

Since the subunit molecular weight of both blood and egg white enzymes is larger than that of both liver and oviduct enzymes, the present result clearly supports our hypothesis that the extracellular NAHase has a higher subunit molecular weight than the intracellular NAHase. Lysosomal enzymes are generally synthesized as precursor with an apparent subunit molecular weight higher than those of mature forms, and precursor-sized enzymes have been shown to be recovered from the extracellular medium.⁴⁻⁸⁾ NAHase might be synthesized as the precursor of that whose subunit molecular weight is about 68000, and then processed to a mature enzyme whose subunit molecular weight is 53000. Studies are now in progress to

ascertain this hypothesis about the biosynthesis of NAHase in this laboratory.

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