Note

Biosynthesis of Chicken β -N-Acetylhexosaminidase: Change from the Precursor to the Secretory Form

Yuji OGAWA and Ryo NAKAMURA

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

Received October 24, 1983

In our previous paper,^{1,2)} we demonstrated that the subunit molecular weight of secretory NAHase of chicken tissues is 68000 and that of thier intracellular enzyme is 53000. Since lysosomal enzymes are generally synthesized as precursors with apparent molecular weights higher than those of mature forms, NAHase of chicken tissues might be synthesized as the precursor of which the subunit molecular weight is about 68000, and then processed to a mature enzyme of which the subunit molecular weight is 53000. We studied the relationship between precursor and secretory forms of this enzyme in either cell-free systems or a *Xenopus* oocyte system.

Total oviduct RNA was extracted with five volumes of 50 mM Tris-HCl, pH 9.0, with 1% (w/v) SDS and an equal volume of 90% (v/v) phenol³⁾ in a Polytron. The aqueous phase was collected after centrifugation of the homogenate at $1500 \times g$ for 15 min and extracted fourth more times with equal volumes of 90% phenol. RNA was precipitated by the addition of two volumes of ethanol followed by incubation at -20°C for 12 hr. The precipitate was collected, dried, and dissolved in water, and the high molecular weight RNA was precipitated from the total RNA by the addition of LiCl to 2 M. After standing for several hours at 0°C , the precipitate was collected, washed twice with 70% (v/v) ethanol to remove residual LiCl, and dissolved in water. The high molecular weight RNA was fractionated into poly(A)⁺ RNA and poly(A)⁻ RNA by oligo [dT]cellulose column chromatography.⁴⁾

The fractionated RNA was precipitated and washed with 70% ethanol, dried under reduced pressure, and then dissolved in a minimal volume of water.

Wheat germ extract was prepared by the procedure of Marcu and Dudock.⁵⁾ The conditions for the translation reactions were optimized by using poly(A)⁺ RNA from oviducts as mRNA, and the following were used: 20 mM Hepes-KOH at pH 7.5, 80 mM CH₃COOK, 0.5 mM MgCl₂, 0.6 mM spermidine, 1 mM ATP, 8 mM creatine phosphate,

creatine kinase (Sigma) at 5 $\mu\text{g}/\text{ml}$, 20 μM GTP, 1 mM dithiothreitol, 19 amino acids (excluding methionine) each at 50 μM , [³⁵S]methionine (1100 Ci/mmol, Amersham) at 11 mCi/ml, and poly(A)⁺ RNA 200 $\mu\text{g}/\text{ml}$.

Rabbit reticulocyte lysate was purchased from New England Nuclear and used under the conditions directed by the supplier.

Oocytes of *Xenopus* were obtained and maintained in modified Barth's⁶⁾ medium. Groups of 30~40 oocytes were microinjected^{7~9)} with 20 ml aliquots of poly(A)⁺ RNA (1.3 mg/ml) with or without tunicamycin at 1 $\mu\text{g}/\text{ml}$. Injected oocytes were cultured for 12 hr at 20°C in unlabeled modified Barth's medium and unhealthy oocytes were then discarded and the remaining oocytes were cultured as batches of 10 in 100 μl of fresh medium, now additionally supplemented with [³⁵S]methionine (40 $\mu\text{Ci}/100 \mu\text{l}$) in Eppendorff microtubes for a further 48 hr. Oocytes and cultured medium were removed and stored at -80°C .

For analysis of the protein synthesized in the *Xenopus* oocytes, the oocytes were homogenized in 500 μl of 0.14 M NaCl, 3 mM KCl, 10 mM sodium phosphate buffer, pH 7.4 supplemented with 1% Triton X-100, 1 mM phenylmethylsulfonylfluoride, and 10 mM of methionine. The homogenates were then allowed to stand for 1 hr and centrifuged for 30 min at $20000 \times g$. The supernatant obtained was analyzed by an immunological procedure.

Anti-NAHase IgG had been prepared previously.²⁾ Translational products of wheat germ extract, rabbit reticulocyte lysate, and *Xenopus* oocytes were dissociated by the addition of SDS at 2% (v/v) and incubated for 3 min. The mixture was made 4% (w/v) with Triton X-100,

FIG. 1. SDS-Polyacrylamide Gel Electrophoretic Pattern of Cell Free Translation Products of NAHase.

Poly(A)⁺ RNA was translated in the wheat germ system.

1: The peptide immunologically related to NAHase antibody.

2: Total products.

FIG. 2. Comparison of Translation Products of Wheat Germ and Reticulocyte Lysate Systems.

- 1: NAHase synthesized in the reticulocyte lysate system.
- 2: NAHase synthesized in the wheat germ system.

diluted with Triton-saline (25 mM Tris-HCl, pH 7.5/0.14 M NaCl/0.1% Triton X-100) and then incubated with 30 μ g of anti-NAHase IgG at 30°C for 1 hr and at 4°C for 12 hr. To isolate the immunoprecipitate, Staphylococcal protein A-Sepharose CL4B (Pharmacia) was added and the incubation was continued for 3 hr with occasional stirring. The Sepharose particles were transferred to a small plastic column and washed successively with a large volumes of Triton-saline and 1 ml of distilled water. The immunoprecipitate bound to protein A-Sepharose was eluted with 60 μ l of sample buffer for SDS polyacrylamide¹⁰⁾ gel electrophoresis and dissociated by incubation at 100°C for 3 min.

Dissociated eluates were subjected to electrophoresis on a slab gel of 12.5% polyacrylamide containing 0.1% SDS with the discontinuous buffer system of Laemmli.¹⁰⁾ After electrophoresis, the gel was stained with Coomassie brilliant blue R-250, destained, and then processed for fluorography using EN³HANCE (New England Nuclear) under the conditions suggested by the manufacture. The dried gel was exposed to a preflashed medical X-ray film (Fuji RX) at -80°C.

As shown in Fig. 1, only one immunoprecipitable polypeptide of molecular weight 65000 was synthesized in the wheat germ system. The same polypeptide was also detected by the reticulocyte lysate system (Fig. 2). These results suggest that NAHases of chicken tissues were synthesized as the same polypeptide whose subunit molecular weight is 65000.

In the *Xenopus* oocytes, the radioactive immunoprecipitable polypeptide of molecular weight 62000 was detected in the cell and that of molecular weight of 68000 was recovered from the medium (Fig. 3). The molecular weight of the peptide which was recovered from the medium coincided with that of secretory NAHase shown in a previous paper.¹⁾ Minor bands (molecular weights, 38000

FIG. 3. NAHase Synthesized in Microinjected *Xenopus* Oocytes.

- 1: ¹⁴C-labeled molecular weight standard proteins.
- 2: Immunoprecipitate from cell fraction which was cultured for 62 hr.
- 3: Immunoprecipitate from medium which was cultured for 62 hr.
- 4: Same conditions as lane 2 except for the addition of tunicamycin.
- 5: Same conditions as lane 3 except for the addition of tunicamycin.
- 6: Immunoprecipitate from cell fraction which was cultured for 62 hr.

and 29000) detectable in lane 2 of Fig. 3 are probably the digested products of synthesized NAHase, because the sum of the molecular weights of these two polypeptides is almost equal to 68000 and no such bands are detectable in the cells when the culture time is short (lane 6). The thick band of molecular weight 43000 detectable in the cell is probably an ovalbumin contaminant. A faint band of which the molecular weight is close to that of a polypeptide in the medium is present in the cell. This band might be an intermediate in the synthesis of secretory NAHase.

A polypeptide band of molecular weight 68000 was not detectable in the medium when tunicamycin, a specific inhibitor of *N*-glycosylation, was added to the culture medium of the oocytes (lane 5 of Fig. 3). This fact indicates the possibility that glycosylation of NAHase is important for its secretion. Hen NAHase might be initially synthesized as the polypeptide of molecular weight 65000, cleaved to 62000 and finally glycosylated to 68000. A part of this enzyme might be secreted as an extracellular enzyme, but large parts of it might be processed to the intracellular enzyme. Although this processing of the protein of M.W. 68000 to the intracellular enzyme could not be done in the *Xenopus* oocyte microinjected system used in this study, the protein might undergo proteolytic processing just the same as other lysosomal enzymes,

including human NAHase.^{11~13)}

REFERENCES

- 1) Y. Ogawa and R. Nakamura, *Agric. Biol. Chem.*, in press (1984).
 - 2) Y. Ogawa, R. Nakamura and Y. Sato, *Agric. Biol. Chem.*, **47**, 2085 (1983).
 - 3) G. Brawerman, *Methods enzymol.*, **30**, 605 (1974).
 - 4) H. Avis and P. Leder, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 1408 (1972).
 - 5) K. Marcu and B. Dudock, *Nucleic Acid. Res.*, **1**, 1385 (1974).
 - 6) L. G. Barth and L. J. Barth, *J. Embryol. Exp. Morph.*, **7**, 210 (1959).
 - 7) C. Lane, S. Shannon and R. Graig, *Eur. J. Biochem.*, **101**, 485 (1979).
 - 8) C. D. Lane, A. Colman, *et al.*, *Eur. J. Biochem.*, **111**, 225 (1980).
 - 9) J. M. Mous, B. L. Peeters, W. J. Heyns and W. A. Rombauts, *J. Biol. Chem.*, **257**, 11822 (1982).
 - 10) U. K. Laemmli, *Nature*, **227**, 680 (1970).
 - 11) A. Hasilic and E. F. Neufeld, *J. Biol. Chem.*, **255**, 4937 (1980).
 - 12) A. Gonzalez-Noriega, J. H. Grubb, V. Talkad and W. S. Sly, *J. Cell Biol.*, **85**, 839 (1980).
 - 13) A. Frisch and E. F. Neufeld, *J. Biol. Chem.*, **256**, 8242 (1981).
-