Vol. 132, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1985 Pages 412-417

INTRACELLULAR TRANSPORT OF HIGH MOLECULAR WEIGHT INTERMEDIATES OF ACID α-GLUCOSIDASE IN HUMAN FIBROBLASTS[+]

Richard D. Henkel[1], Ellen Richie[2] and Julian C. Williams[*]

Metabolic Disease Division
Department of Pediatrics
University of Texas Medical School at Houston
6431 Fannin
Houston, TX 77030

Received August 28, 1985

SUMMARY: Two transient, high molecular weight precursors of human acid α-glucosidase were detected by immune precipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The high molecular weight precursors were rapidly converted into lower molecular weight forms corresponding to previously identified intermediates of acid α-glucosidase. An accumulation of these precursors was observed in fibroblasts treated with monesin and nigericin, suggesting that these precursors are intermediates of acid α-glucosidase undergoing transport through the Golgi complex.

Lysosomal enzymes are synthesized as high molecular weight precursors on membrane bound ribosomes and inserted into the lumen of the endoplasmic reticulum. Glycosylation occurs during transfer across the microsomal membrane (1,2) before transport to the Golgi complex. The carbohydrate chains of lysosomal enzyme precursors are phosphorylated and processed during transport through the Golgi apparatus (3,4). In addition to carbohydrate modification, most lysosomal enzymes undergo proteolytic processing during maturation (1,5,6). Previous studies have shown that a high molecular weight precursor of acid α-glucosidase (EC 3.2.1.20) containing a 109 kDa polypeptide chain is converted through a series of intermediates into low-uptake forms of enzyme found in the lysosome (8,9).

In this report, we describe several processing steps in the maturation of human acid α-glucosidase that occur prior to the formation of the 109 kDa precursor of this enzyme.

[+]Supported in part by grant 83-781 from the American Heart Association.
[1]Present address: Virology and Immunology Department, Southwest Foundation for Biomedical Research, San Antonio, TX 78284.
[2]Present address: University of Texas System Cancer Center, Science Park. Research Division, P. O. Box 389, Smithville, TX 78957.
[*]To whom requests for reprints should be addressed.

0006-291X/85 $1.50

MATERIALS AND METHODS

Purification of human hepatic acid α-glucosidase

Acid α-glucosidase was purified from human liver by ammonium sulfate precipitation, Sephadex G-100 chromatography, and AcA-44 gel filtration chromatography as previously described (9,10). Purified hepatic α-glucosidase was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), native polyacrylamide gel electrophoresis, and by isoelectric focusing as previously described (10).

Preparation of antisera

Rabbit antiserum against human hepatic acid α-glucosidase was prepared by inoculation of 2 mg doses of purified hepatic acid α-glucosidase emulsified in complete Freund's adjuvant. Doses were administered biweekly via footpad or intramuscular injections. Blood was collected from lateral ear veins prior to and at various intervals after immunization. Serum was separated after clotting and stored in aliquots at -20°C.

Radioactive labeling of fibroblast acid α-glucosidase

Normal human fibroblasts were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 20% fetal bovine serum, (FBS, Irvine Scientific), and 2 mM glutamine before labeling. Confluent fibroblast monolayers in 35 mm plastic petri dishes were prepared for labeling by washing cells twice with isotonic 100 mM Tris buffered saline (TBS), pH 7.2, followed by a 30 minute incubation period in medium lacking amino acids. Cells were pulse-labeled for various lengths of time with [^{14}C]-labeled amino acids (Amersham) in DMEM supplemented with 3% dialyzed FBS. After labeling, cells were washed twice with TBS and either chased for various lengths of time in maintenance medium or harvested immediately with 0.25% trypsin and 0.04% EDTA. Cell cultures were harvested after the chase period as described above. Harvested cells were collected by centrifugation, washed twice with TBS and disrupted by sonication. Fibroblast lysates were solubilized in disruption buffer (TBS containing 1% NP-40 and 1% Aprotinin) and stored at -20°C.

Fibroblasts were incubated with monensin and nigericin (Sigma) at concentrations of 3 μM and 0.5 μM, respectively, for 1 hour prior to and throughout the pulse-chase period, then labeled and harvested in a manner identical to that previously described for pulse-chase experiments.

Immune precipitation

Acid α-glucosidase was immune precipitated from radioactively labeled fibroblast lysates with rabbit antiserum by mixing 50 μl of radioactively labeled lysate with 25 μl of undiluted rabbit anti-acid α-glucosidase serum or control serum and then incubating this mixture for 12 hours at 4°C. Next, 100 μl of a 10% (w/v) suspension of formalin-fixed Staphylococcus aureus (Calbiochem-Behring) was added to each sample and incubated for 30 minutes at 22°C. Immune complexes absorbed to the bacterial suspension were collected by centrifugation in a Brinkman microfuge for 5 minutes. Immune complexes were washed three times with disruption buffer supplemented with 1% sodium deoxycholate and 0.1% sodium dodecyl sulfate. Immune complexes were denatured by boiling the washed pellets for 3 minutes in 10 mM Tris-HCl, pH 6.8, containing 4% sodium dodecyl sulfate, 4% 2-mercaptoethanol, and 20% glycerol. Acid α-glucosidase was also precipitated with mouse monoclonal anti-human acid α-glucosidase antibodies by mixing 50 μl of radioactively labeled fibroblast lysate with 150 μg of purified IgM monoclonal antibody or normal mouse IgM for 12 hours at 4°C. Immune complexes were precipitated by adding 300 μg of affinity purified goat anti-mouse IgM for 2 hours at 4°C followed by

centrifugation in a Brinkman microfuge for 15 minutes. Precipitates were washed twice with disruption buffer before denaturation as described above.

Immune precipitates were analyzed by SDS-PAGE in 6% slab gels as described by Laemmli (11). After electrophoresis, gels were fixed for 1 hour in 25% methanol and 7% acetic acid. Fixed gels were washed in distilled water for 30 minutes, then soaked in Autofluor (National Diagnostics) for 1 hour before drying at 60°C under vacuum. Autoradiographs were made by exposing dried gels to Kodak X-OMAT film at -70°C. Electrophoretic mobilities of immune precipitated polypeptides were compared with [^{14}C]-labeled molecular weight markers (Amersham). Molecular weights of polypeptides were estimated with semi-logarithmic plots of relative mobility versus molecular weight (11).

RESULTS AND DISCUSSION

The biosynthesis of acid α-glucosidase was characterized by immune precipitation of the enzyme from fibroblasts labeled with [^{14}C]-amino acids. Results of pulse-chase experiments wherein fibroblasts were labeled for 30 minutes and chased for various lengths of time up to 6 hours after labeling are shown in Figure 1. Acid α-glucosidase in fibroblasts harvested between 0 and 60 minutes after labeling and immune precipitated with rabbit anti-human hepatic acid α-glucosidase antibodies contained 135 kDa, 122 kDa, and 112 kDa polypeptides when analyzed by SDS-PAGE (Figure 1, lanes A-C). The molecular weight of radioactively labeled polypeptides in the immune precipitates decreased as the chase period increased. The major polypeptide immune precipitated between 1.5 and 3 hours after labeling was 112 kDa (Figure 1, lanes

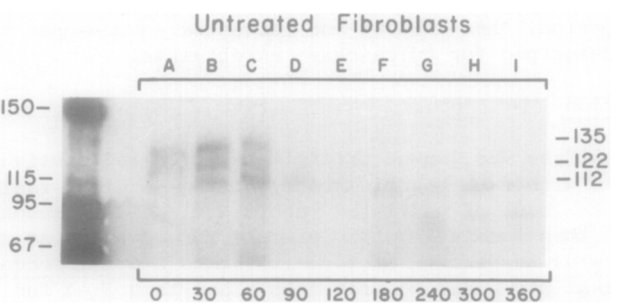

Figure 1: Biosynthesis of acid α-glucosidase. The biosynthesis of human acid α-glucosidase was characterized by immune precipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Fibroblasts were labeled for 30 minutes by incorporation of [^{14}C]-amino acids and chased for various lengths of time prior to incubation with rabbit antiserum raised against human hepatic acid α-glucosidase. The electrophoretic mobilities and molecular weights in kilodaltons of [^{14}C]-labeled protein standards are shown in the lane on the left side of the gel. Lanes A through I illustrate the polypeptide chains immune precipitated from fibroblasts chased various lengths of time after labeling. The numbers shown below each lane indicate the length of time in minutes that the pulse-labeled fibroblasts were chased after labeling.

D-F) and between 4 and 6 hours after labeling both 112 kDa and 95 kDa peptides were detected (lanes G-I).

Immune precipitates collected after a 90 minute chase period (Figure 1, lanes D-F) contained polypeptides that are similar to several biosynthetic forms of acid α-glucosidase that have been reported previously (7,8,12). A 109 kDa precursor of acid α-glucosidase has been isolated from human urine and fibroblast tissue culture supernatants (7,12). The 112 kDa polypeptide observed in Figure 1 corresponds to the previously reported 109 kDa precursor within the margin of error associated with molecular weight estimation in polyacrylamide gels. This 112 kDa precursor was observed prior to the appearance of a 95 kDa intermediate of acid α-glucosidase. Haslik and Neufeld have described a 95 kDa precursor of acid α-glucosidase that undergoes proteolytic processing into lower molecular weight forms of enzyme (8).

The transient polypeptides with molecular weights greater than 112 K detected within 60 minutes after labeling (Figure 1, lanes A-C) were also

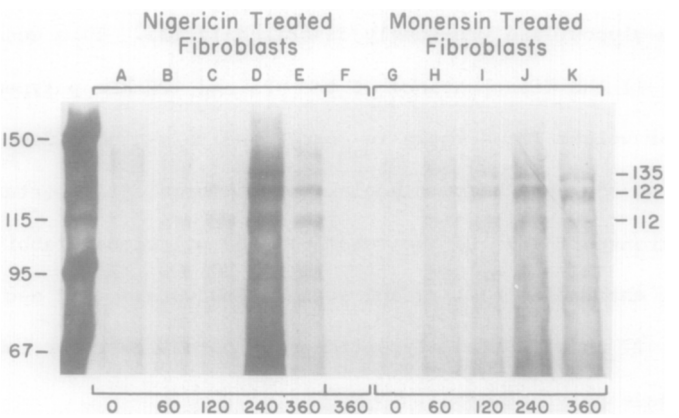

Figure 2: Inhibition of acid α-glucosidase transport in human fibroblasts. The transport of acid α-glucosidase in fibroblasts was characterized by blocking the egress of newly synthesized proteins from the Golgi complex with monensin and nigericin. Fibroblast cultures were biosythetically labeled in the presence of 0.5 μM and 3 μM concentrations of nigericin and monensin, respectively, for 60 minutes and chased for various lengths of time before harvesting the cells. Acid α-glucosidase synthesized and processed in the presence of these agents was immune precipitated with antisera raised against hepatic acid α-glucosidase and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Numerical values indicated on the margins represent the relative molecular mass of the polypeptides immune precipitated by rabbit antiserum from fibroblasts treated with monensin and nigericin for the lengths of time in minutes indicated below each lane. Lane F contains the polypeptides immune precipitated from nigericin treated fibroblasts after a 360 minute chase period by rabbit serum collected prior to immunization with hepatic acid α-glucosidase.

observed in immune precipitates from fibroblasts treated with monensin and nigericin (Figure 2). These agents have been demonstrated to inhibit glycoprotein transport from the Golgi apparatus (13-16). Monensin is believed to block the transport of glycoproteins from the cis to the trans region of the Golgi complex (13,15,16). Nigericin inhibits transport of protein from the trans cisternae (14). Immune precipitates from fibroblasts treated with monensin contained primarily 135 and 122 kDa polypeptides after a 6 hour chase period whereas 122 and 112 kDa chains accumulated in fibroblasts treated with nigericin (Figure 2, lanes A-E and G-K, respectively). The distribution of the radioactive polypeptides in fibroblasts treated with these agents suggests that the formation of the 112 kDa polypeptide occurs in the trans subcompartment of the Golgi apparatus.

These results indicate that newly synthesized acid α-glucosidase undergoes several rapid transformations during transport through the Golgi complex in fibroblasts. We conclude that the 135 and 122 kDa polypeptides observed in the experiments described above are biosynthetic precursors of the 109 kDa form of acid α-glucosidase previously described (7,12). This conclusion is supported by: 1) the disappearance of the 135 and 122 kDa polypeptides as lower molecular weight forms known to correspond to α-glucosidase appear, 2) the accumulation of these high molecular weight forms after perturbation of glycoprotein transport and, 3) the reactivity of polyclonal rabbit antisera and monoclonal antibodies (17) raised against low uptake acid α-glucosidase with the 135, 122 and 112 kDa polypeptides indicates the occurrence of common epitopes on these polypeptides.

REFERENCES

1. Erickson, A. H., Conner, G. E., and Blobel, G. (1981) J. Biol. Chem. 256, 11224-11231.
2. Rosenfeld, M.G., Kreibich, G., Popov, D., Kato, K., and Sabatini, D.D. (1982) J. Cell Biol. 93, 135-143.
3. Waheed, A., Haslik, A., and von Figura, K. (1981) J. Biol. Chem. 256, 5717-5721.
4. Pohlmann, R., Waheed, A., Haslik, A., and von Figura, K. (1982) J. Biol. Chem. 257, 5323-5325.
5. Schuchman, E. H., Guzman, N. A., and Desnick, R. J. (1984) J. Biol. Chem. 259, 3132-3140.
6. Frisch, A. and Neufeld, E. F. (1981) J. Biol. Chem. 256, 8242-8246.

7. Oude-Elferink, R.P.J., Brouwer-Kelder, E.M., Surya, I., Strijland, A., Kroos, M., Reuser, A.J.J., and Tager, J.M. (1984) Eur. J. Biochem. 139, 489-495.
8. Haslik, A. and Neufeld, E.F. (1980) J. Biol. Chem. 255, 4937-4945.
9. Chambers, J.P. and Williams, J.C. (1983) Enzyme 29, 109-119.
10. Henkel, R. D., Chambers, J. P., and Williams, J. C. (1985) Biochim. Biophys. Acta, 829, 44-50.
11. Laemmli, U. K. (1970) Nature 227, 680-685.
12. Oude-Elferink, R.P.J., Strijland, A., Surya, I., Brouwer-Kelder, E.M., Kroos, M., Hilkens, J., Hilgers, J., Reuser, A.J.J., and Tager, J.M. (1984) Eur. J. Biochem. 139, 497-502.
13. Tartakoff, A.M. (1982) Phil. Trans. R. Soc. Lond. B300, 173-184.
14. Vladutiu, G. D. (1984) Biochem. J. 218, 261-268.
15. Griffiths, G., Quinn, P., and Warren, G. (1983). J. Cell Biol. 96, 835-850.
16. Quinn, P., Griffiths, G., and Warren, G. (1983). J. Cell Biol. 96, 851-856.
17. Henkel, R. D., Dreesman, G. R., Kennedy, R. C., Howell, R. R., and Williams, J. C. (1985) Hybridoma, in press.