Characterization and Immunolocalization of Bovine N-Acetylglucosamine-1-phosphodiester α-N-Acetylgulosaminidase*

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N-Acetylglucosamine-1-phosphodiester α-N-acetylgulosaminidase (phosphodiester α-GlcNAcase) has been purified 3,000-fold from bovine liver and its kinetic properties determined as described in the previous report (Mullis, K. G., Huynh, M., and Kornfeld, R. (1993) J. Biol. Chem. 268, 1718–1726). This report describes the hydrodynamic and lectin binding properties of phosphodiester α-GlcNAcase as well as its intracellular localization. The molecular weight of phosphodiester α-GlcNAcase is 204,950, as determined from density gradient centrifugation in D₂O and H₂O glycerol gradients and gel filtration. Enzymatically active enzyme migrates on sodium dodecyl sulfate-polyacrylamide gel electrophoresis with a molecular weight of 128,000, consistent with native phosphodiester α-GlcNAcase being a dimer.

The lectin binding properties of phosphodiester α-GlcNAcase indicate that it contains sialylated species of both complex type N-linked oligosaccharides and O-linked oligosaccharides. In immunofluorescence studies phosphodiester α-GlcNAcase shows a perinuclear, Golgi localization in Vero cells as does the mid-Golgi marker α-mannosidase II. After exposure of the Vero cells to brefeldin A, phosphodiester α-GlcNAcase assumes an endoplasmic reticulum staining pattern. In contrast, in cells costained with the trans-Golgi marker wheat germ agglutinin, the wheat germ agglutinin marker assumed an endosomal network appearance after exposure to brefeldin A. These findings indicate that phosphodiester α-GlcNAcase is normally located within the Golgi stack, separate from the trans-Golgi and trans-Golgi network stained by wheat germ agglutinin.

In the preceding paper (1) we described the 3,000-fold purification of N-acetylglucosamine-1-phosphodiester α-N-acetylgulosaminidase (phosphodiester α-GlcNAcase) from bovine liver. This enzyme catalyzes the second step in the formation of the mannose 6-phosphate recognition marker on lysosomal enzyme oligosaccharides by removing the N-acetylglucosamine residue from the GlcNAc-P-mannose formed by GlcNAc phosphotransferase action. The purification of bovine liver phosphodiester α-GlcNAcase has enabled us to characterize the enzyme biochemically with regard to its catalytic and kinetic properties, as described in the preceding report (1) and also its physical properties as described in this report. The molecular weight of the phosphodiester α-GlcNAcase was determined by analysis of its hydrodynamic properties, and its oligosaccharide composition was characterized using plant lectins.

The intracellular localization of phosphodiester α-GlcNAcase activity has been analyzed previously by kinetic studies in intact cells and by subcellular fractionation techniques. In pulse-chase labeling studies performed in transport-impaired mouse lymphoma cells, Lazarrino and Gabel (2) showed that the initial phosphorylation of lysosomal enzyme oligosaccharides occurred in a compartment proximal to that in which they were uncovered by phosphodiester α-GlcNAcase. Goldberg and Kornfeld (3) demonstrated that when mouse lymphoma cell membranes are fractionated on sucrose gradients, phosphodiester α-GlcNAcase activity fractionates with a lighter compartment than GlcNAc-phosphotransf erase and clearly distinct from galactosyltransferase activity found in a much lighter fraction. In a similar study, Schweizer et al. (4) showed that in extracts from Vero cells, phosphodiester α-GlcNAcase activity cofractionates with GlcNAc-phosphotransferase activity at a density intermediate between the pre-Golgi marker, p53 and galactosyltransferase. These data indicate, albeit indirectly, that phosphodiester α-GlcNAcase most likely resides in the cis-/medial-Golgi. We have generated and used a monoclonal antibody directed against phosphodiester α-GlcNAcase to visualize directly the intracellular location of phosphodiester α-GlcNAcase by immunofluorescence studies. We have used brefeldin A, a fungal metabolite, to define further the intracellular localization of phosphodiester α-GlcNAcase within the Golgi.

EXPERIMENTAL PROCEDURES

MATERIALS—Partially purified phosphodiester α-GlcNAcase was prepared from bovine liver as described previously (1). Methyl α-D-mannopyranoside, quaternary aminoethyl Sephadex (QAE-Sephadex), and Lubrol-PX were from Sigma. Uridine diphosphate-[glucosamine,6-3H]N-acetyl-d-glucosamine (UDP-[3H]GlcNAc, 25 Ci/mM) was from American Radiolabeled Chemicals (St. Louis). ScintiVerse I was from Fisher. Superoxide 6 and the protein standards used for gel filtration (thioglycolulin, ferritin, and catalase) were obtained from Pharmacia LKB Biotechnology Inc. Protein standards used in glycerol density gradients were obtained from Sigma. Vibrio cholerae neuraminidase was obtained from Calbiochem. Prestained protein standards and nitrocellulose were obtained from Bio-Rad. The following digoxigenin-labeled lectins: Galanthus nivalis agglutinin, Sambu-
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cus nigra agglutinin, Maackia amurensis agglutinin, peanut agglutinin (PNA), and Datura stramonium agglutinin, as well as alkaline phosphatase-labeled anti-digoxigenin antibody, fetuin, asialofetuin, and blocking reagent were obtained in the glycan detection kit from Boehringer Mannheim. Western Blue was obtained from Promega.

Reduced and denatured phosphodiester α-GlcNAcase was obtained from Jackson Laboratories. Vero cells were obtained from ATCC. Brefeldin A was obtained from Sigma and was prepared as a 10 mg/ml stock in ethanol, stored at -20 °C. Labtek four-chamber permanox slides were obtained from Nunc. FITC-labeled donkey anti-mouse IgM and FITC-labeled donkey anti-rabbit IgG were obtained from Jackson ImmunoResearch Laboratories. Texas red-labeled wheat germ agglutinin (WGA) was obtained by VE Laboratories. All other chemicals were analytical grade.

Buffers—The following buffers were used. Tris-buffered saline was 50 mM Tris, pH 7.5, 150 mM NaCl. Buffer 1 was 50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM CaCl₂, 1 mM MgCl₂, 1 mM MnCl₂. PBS was 140 mM NaCl, 2.7 mM KCl, 10 mM NaHPO₄, 1.8 mM KH₂PO₄, pH 7.5.

Synthesis of [3H]GlcNAc-a-P-Man-a-Me—[3H]GlcNAc-a-P-Man-a-Me was synthesized from UDP-[3H]GlcNAc, using methyl α-d-mannopyranoside and GlcNAc-phosphotransferase from Amoeba castellanei as described previously by Mullis and Ketcham (5).

Assay of Bovine Phosphodiester α-GlcNAcase—Phosphodiester α-GlcNAcase was incubated for 1 h at 37 °C in a reaction mixture of 0.03 ml containing 50 mM Tris maleate, pH 6.7, 0.5% (v/v) Triton X-100, and 1 mM [3H]GlcNAc-a-P-Man-a-Me, and the reaction products were analyzed by ion exchange chromatography on QAE-Sephadex as described previously (5).

Gel Filtration Chromatography and Determination of Stokes Radii—Gel filtration chromatography of pooled phosphodiester α-GlcNAcase eluted from WGA-Sepharose was performed using Superose 6 as described previously (1). The Stokes radius of bovine phosphodiester α-GlcNAcase was determined by the method of Ackers and Lane (10).

SDS-Polyacrylamide Gel Electrophoresis—SDS-polyacrylamide gel electrophoresis was carried out following the procedures of Laemmli (4). Rabbits were immunized in medium 199 containing 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml fungizone. Eighteen to 24 h prior to immunofluorescence, a 100-fold dilution of ascites fluid in 1:100 dilution of mouse anti-phosphodiester α-GlcNAcase 5B6.3 ascites fluid in 10% donkey serum/PBS, a 1:1,000 dilution of rabbit anti-α-mannosidase II in 10% donkey serum/PBS, or a 1:50 dilution of Texas red-labeled WGA (WGA-TR) in 10% donkey serum/PBS. The cells were washed three times for 5 min with PBS, then fixed/permeabilized for 2 min with ice-cold acetone. The cells were washed twice with PBS for 5 min and blocked for 1 h with 10% donkey serum/PBS. The slides were incubated for 1 h with either a 1:100 dilution of mouse anti-phosphodiester α-GlcNAcase 5B6.3 ascites fluid in 10% donkey serum/PBS, a 1:100 dilution of mouse anti-phosphodiester α-GlcNAcase 5B6.3 ascites fluid in 10% donkey serum/PBS, and then incubated in secondary antibody for 1 h with shaking (secondary antibody: 1:50 FITC-labeled donkey anti-mouse IgM or 1:50 FITC-labeled donkey anti-rabbit IgG). The cells were washed three times for 5 min with 10% donkey serum/PBS. The slides were overlaid with 50% glycerol (v/v), PBS containing 0.1 M n-propyl gallate, and VWR No. 1 overcups (24 x 60 mm) were mounted. Confocal microscopy was performed using a 60 x objective lens, and photomicrographs were taken using Kodak TMAX ASA 400 film.

In the colocalization studies, cells were prepared essentially as described above. Cells were incubated for 1 h with a 1:50 dilution of WGA-TR in 10% donkey serum/PBS. The cells were washed three times with 5% Tween 80/2% donkey serum/PBS, incubated for 1 h in a 1:100 dilution of mouse anti-phosphodiester α-GlcNAcase 5B6.3 ascites fluid, and washed three times for 5 min in 10% donkey/PBS before incubation for 1 h in a 1:50 dilution of FITC-labeled donkey anti-mouse IgM secondary antibody. The cells were washed three times for 5 min in 10% donkey serum/PBS. The slides were overlaid with 50% glycerol (v/v), PBS containing 0.1 M n-propyl gallate, and VWR No. 1 coverslips (24 x 60 mm) were mounted. Confocal microscopy was performed using an MRC-500 scanning laser confocal microscope (Bio-Rad). Photographs of images from video monitors were recorded on Tmax 100 ASA film.

RESULTS

In the accompanying report (1) the general properties, substrate specificity, and kinetic parameters of purified bovine phosphodiester α-GlcNAcase were described. In this report a number of its physical and hydrodynamic properties as well as its subcellular localization are presented.

Hydrodynamic Analysis of Bovine Phosphodiester α-GlcNAcase—Phosphodiester α-GlcNAcase has a molecular weight of 129,000 as determined by its migration in nonreducing SDS-polyacrylamide gel electrophoresis (Table I). However, when subjected to gel filtration on Sephadex G-75, the enzyme eluted near the ferritin standard with a M₅₀ equivalent of 400,000, corresponding to a Stokes radius of 70.75 Å (Fig. 1). The diffusion coefficient (Dₑ) calculated from these data is 3.07 x 10⁻⁶ cm²/s.

Since phosphodiester α-GlcNAcase is a membrane-bound glycoprotein requiring detergent for solubilization, we performed hydrodynamic analysis to determine the amount of detergent that is bound to the enzyme. Bound detergent causes a marked shift in apparent sedimentation in solvents of different densities (11). Fig. 2 shows the results obtained when partially purified phosphodiester α-GlcNAcase was subjected to density gradient centrifugation in 9–40% glycerol in either H₂O (panel A) or D₂O (panel B). In both gradients, phosphodiester α-GlcNAcase sedimented between the malate and sucrose limits. The relative sedimentation coefficient of phosphodiester α-GlcNAcase was 6.4 S in H₂O and 5.4 S in D₂O. When the values were corrected to H₂O at 20 °C, the sedimentation coefficient of phosphodiester α-GlcNAcase was 7.4 S in both gradients. These data indicated that phosphodiester α-GlcNAcase binds...
The fact that the enzyme bound to concanavalin A-Sepharose contained either sialic acids or GlcNAc residues. To define the types of oligosaccharides on phosphodiester α-GlcNAcase, the enzyme was subjected to SDS-polyacrylamide gel electrophoresis, blotted onto nitrocellulose, and the blots were probed with five plant lectins of different carbohydrate binding specificities. As shown in Fig. 3 and Table II, phosphodiester α-GlcNAcase was reactive with the Galβ1,4GlcNAc binding lectin D. stramonium agglutinin and the sialic acid binding lectins M. amurensis agglutinin and S. nigra agglutinin. When the enzyme was digested prior to SDS-polyacrylamide gel electrophoresis with V. cholerae neuraminidase, which removes sialic acids, it lost reactivity with M. amurensis agglutinin and S. nigra agglutinin, retained reactivity with D. stramonium agglutinin, and gained reactivity with PNA (Fig. 3). Table II shows the carbohydrate binding specificity of the lectins used and presents a summary of the lectin reactivities of phosphodiester α-GlcNAcase. The acquisition of reactivity to PNA, which recognizes Galβ1,3GalNAc linkages typical of O-linked oligosaccharides, indicates that the enzyme contains a sialylated form of the O-linked disaccharide. The fact that S. nigra agglutinin binds to phosphodiester α-GlcNAcase shows that the enzyme contains sialic acid α2,6GalNAc linkages on its O-linked chains. G. nivalis agglutinin does not react with phosphodiester α-GlcNAcase, indicating that terminal mannose residues found on high mannose oligosaccharides are not present. D. stramonium agglutinin, which recognizes Galβ1,4GlcNAc, an underlying structure in complex type N-linked oligosaccharides, reacts with phosphodiester α-GlcNAcase. However, when phosphodiester α-GlcNAcase was first treated with N-glycanase, to remove the N-linked oligosaccharides, binding of D. stramonium agglutinin was abolished (data not shown). Phosphodiester α-GlcNAcase is bound by M. amurensis agglutinin, which recognizes sialic acid α2,3Gal residues on complex type N-linked and perhaps O-linked oligosaccharides. Taken together these data suggest that phosphodiester α-GlcNAcase contains complex type asparagine-linked oligosaccharides and O-linked oligosaccharides as shown in Table II. These data are in agreement with the lectin affinities observed during purification of bovine phosphodiester α-GlcNAcase (1).

Bovine Phosphodiester α-GlcNAcase Is Localized to the Golgi Apparatus—To define more precisely the intracellular localization of phosphodiester α-GlcNAcase, we used a mouse monoclonal antibody directed against the native enzyme in immunofluorescence studies. Table III shows that the mouse monoclonal antibody 5B6.3, which is an IgM, immunoprecipitated phosphodiester α-GlcNAcase activity, whereas an irrelevant mouse monoclonal antibody did not. In both human fibroblasts (data not shown) and Vero cells, phosphodiester α-GlcNAcase is localized in a juxtanuclear region of the cells as shown in the lower right panel of Fig. 4. The lower left...
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FIG. 2. Hydrodynamic properties of phosphodiester α-GlcNAcase.
Eleven units of partially purified bovine phosphodiester α-GlcNAcase were layered onto 5 ml of 9–40% glycerol density gradients prepared in D₂O or H₂O and centrifuged in an SW 65 rotor at 60,000 rpm for 16 h (H₂O gradient, panel A) or 24 h (D₂O gradient, panel B). Gradients were fractionated into 33 samples (150 μl); fractionation is shown from top (left) to bottom (right), and the fractions were assayed for phosphodiester α-GlcNAcase activity (○), as described under "Experimental Procedures." The sedimentation standards were: 1, cytochrome c (2.15 S); 2, ovalbumin (3.6 S); 3, malate dehydrogenase (4.35 S); 4, lactate dehydrogenase (7.35 S); 5, catalase (11.35 S). The relative sedimentation coefficient of phosphodiester α-GlcNAcase was 5.4 S in D₂O and 6.4 S in H₂O.

panel of Fig. 4 shows that α-mannnosidase II, an enzyme of the medial-Golgi, has a similar juxtanuclear localization in Vero cells.

WGA binds to highly sialylated glycoproteins present in the trans-Golgi and trans-Golgi network (TGN). WGA-TR staining in Vero cells is juxtanuclear but has a much tighter localization than phosphodiester α-GlcNAcase as shown in the left panels of Fig. 5. In cells labeled for both phosphodiester α-GlcNAcase and WGA-TR, there are areas of overlap, but the staining of WGA-TR and the localization of phosphodiester α-GlcNAcase are not identical as shown in the bottom left panel of Fig. 5.

The right panels of Fig. 5 show the results obtained when cells were exposed to 10 μg/ml brefeldin A (BFA) for 15 min before fixation and permeabilization. Phosphodiester α-GlcNAcase disperses into an ER pattern after BFA treatment consistent with its localization in the Golgi stacks (12). WGA staining in BFA-treated Vero cells is less dispersed than the staining seen for phosphodiester α-GlcNAcase. In cells both labeled for phosphodiester α-GlcNAcase and stained for
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Fig. 3. Lectin blot analysis of phosphodiester α-GlcNAcase. 3,000-fold purified phosphodiester α-GlcNAcase was digested with neuraminidase (+) or not (−) as described under “Experimental Procedures.” Aliquots of phosphodiester α-GlcNAcase and either fetuin (F) or desialyzed fetuin (dF) were subjected to electrophoresis on 7.5% SDS-polyacrylamide and transferred to nitrocellulose as described under “Experimental Procedures.” Blots were probed with the following digoxigenin-labeled lectins: S. nigra agglutinin (SNA), M. amurenensis agglutinin (MAA), PNA, or D. stramonium agglutinin (DSA). The bound lectins were visualized with alkaline phosphatase-coupled antidigoxigenin. The positions of prestained standard proteins of the indicated molecular mass in kDa are shown on the right of each panel. PGase denotes the position of phosphodiester α-GlcNAcase.

### TABLE II

Summary of the lectin blot analysis

Experiments corresponding to the lectin blot analysis are shown in Fig. 3. Positive (+) indicates reactivity as defined by the presence of a phosphodiester α-GlcNAcase (PGase) band after probing with digoxigenin-labeled lectins and alkaline phosphatase-labeled anti-digoxigenin IgG. Negative (−) results are defined as lack of phosphodiester α-GlcNAcase band. The two carbohydrate structures shown are possible structures based on the composition defined by the lectin blot analysis, but the exact carbohydrate structures cannot be ascertained from these data. SA, sialic acid.

| Carbohydrate Structure | SNA | MAA | PNA | DSA |
|------------------------|-----|-----|-----|-----|
| PGase                  | −   | +   | −   | −   |
| PGase + N-glycanase    | −   | +   | ±   | −   |
| PGase + neuraminidase  | +   | −   | −   | −   |

SA α2,3 Gal-GalNAc-Ser/Thr | α2,6
SA

### TABLE III

Immunoprecipitation of phosphodiester α-GlcNAcase activity

Reaction mixtures containing 4 μl of phosphodiester α-GlcNAcase activity (0.19 nmol/h) and 100 μl of tissue culture supernatant were incubated on ice for 30 min. Rabbit anti-mouse secondary antibody (2 μl) was then added to the immune complex and incubated for 30 min. The entire complex was precipitated using staphylococcal A cells, and 3 × washed pellets were directly assayed for phosphodiester α-GlcNAcase activity.

| Tissue culture supernatant | Activity in immune complex (nmol/h) |
|---------------------------|------------------------------------|
| 5B6.3                     | 0.11                               |
| 5B6.4 (irrelevant clone)  | 0                                  |

WGA-TR, the pattern of staining is not coincident and is suggestive of two different networks.

### DISCUSSION

The hydrodynamic studies of phosphodiester α-GlcNAcase have revealed that this membrane-bound glycoprotein has a calculated molecular weight of 204,550 as derived from the Svedberg equation. Many detergent-solubilized membrane proteins are isolated as protein-detergent complexes containing substantial amounts of detergent which causes the complex to have a high partial specific volume compared with standard globular proteins. Therefore, we subjected the bovine liver phosphodiester α-GlcNAcase to density gradient centrifugation in both D₂O and H₂O to derive values for the partial specific volume, 5, and the sedimentation coefficient s₂₀,₀ of the enzyme-detergent complex. If a protein-detergent
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**FIG. 4.** Localization of phosphodiester α-GlcNAcase and α-mannosidase II by indirect immunofluorescence. Vero cells were prepared as described under "Experimental Procedures." Phosphodiester α-GlcNAcase was detected using mouse anti-native phosphodiester α-GlcNAcase monoclonal antibody 5B6.3 and FITC-conjugated donkey anti-mouse IgM secondary antibody (lower right). α-Mannosidase II was detected using rabbit anti-native α-mannosidase II polyclonal antibodies and FITC-conjugated goat anti-rabbit IgG secondary antibody (lower left). The upper panels show cells that were reacted with FITC-conjugated secondary antibodies alone. Fluorescence was viewed by indirect immunofluorescence.

**FIG. 5.** Effect of BFA on the Golgi localization of phosphodiester α-GlcNAcase and the TGN localization of WGA staining. Vero cells were treated (+) or not (−) with BFA (10 μg/ml) for 15 min at 37 °C, the cells were fixed and permeabilized with ice-cold acetone, and phosphodiester α-GlcNAcase was detected using anti-phosphodiester α-GlcNAcase monoclonal antibody 5B6.3 and FITC-conjugated donkey anti-mouse IgM secondary antibody, as described under "Experimental Procedures." Control panels were treated only with secondary antibody. Other cells, prepared as described under "Experimental Procedures," were stained using WGA-TR. Cells in the two bottom panels were colabeled for phosphodiester α-GlcNAcase and WGA-TR. FITC staining is shown on the left; Texas red staining is shown on the right. The fluorescence was viewed by confocal microscopy.

Complex contains substantial detergent it will sediment to a "lighter" position of a D₂O gradient compared with its position on an H₂O gradient relative to standard proteins (11). The phosphodiester α-GlcNAcase sedimented in the same position relative to the standard proteins in both the D₂O and H₂O gradients indicating that the enzyme binds little detergent, and the value of its sedimentation coefficient was the same in both gradients when corrected to water at 20 °C. The value for the partial specific volume of phosphodiester α-GlcNAcase calculated from density gradient centrifugation is 0.7142 cm³/g. This value is similar to the partial specific volumes of the protein standards used in the density gradient centrifugation which range from 0.72–0.75 cm³/g and much lower than the partial specific volume of 0.958 cm³/g for the detergent Lubrol-PX.

The Stokes radius for phosphodiester α-GlcNAcase was determined using gel filtration of the enzyme in comparison with globular protein standards of known molecular weight and Stokes radius. The enzyme has a Stokes radius of 70.7 Å, a value that is inconsistent for a globular protein with a molecular weight of 204,550, which should have a Stokes radius of approximately 45 Å. The frictional ratio (f/f₀), calculated for phosphodiester α-GlcNAcase from the values for its molecular weight, partial specific volume, and Stokes radius, is 1.8, which indicates that the molecule is significantly more asymmetric than typical globular proteins which have frictional ratios of 1–1.3. Therefore, we propose that phosphodiester α-GlcNAcase is not globular, but instead has an extended structure, the exact nature of which cannot be elucidated from the data given here.

In contrast to the molecular weight of 204,550 derived from hydrodynamic measurements, enzymatically active phosphodiester α-GlcNAcase migrates on SDS-polyacrylamide gel electrophoresis like a protein of molecular weight 129,000. This observation suggests that native enzyme may be a dimer that dissociates into monomers in SDS. Since the enzyme eluted from the SDS-gels is active, either the monomer is enzymatically active or may be able to reform dimers under the elution conditions used to extract enzymatic activity. We are unable to distinguish between these two possibilities. It is interesting that Ben-Yoseph et al. (13), using the method of radiation inactivation, report a molecular size of 129 kDa for human placental phosphodiester α-GlcNAcase.

In the purification of bovine liver phosphodiester α-GlcNAcase, enzyme molecules with carbohydrates containing asparagine-linked oligosaccharides and sialic acid were selected because of the binding affinities of concanavalin A and WGA. Those enzyme molecules that do not bind to these lectins because they lack the necessary carbohydrate epitopes constitute 26–40% of the total enzyme activity in different preparations (1). The lectin binding experiments allowed us to examine the carbohydrate composition of the glycosylated phosphodiester α-GlcNAcase further. The enzyme bound not only to concanavalin A, but also to M. amurensis agglutinin and D. stramonium agglutinin, although not G. nivalis agglutinin, a finding consistent with the presence of complex type N-linked oligosaccharides. The enzyme also bound WGA and S. nigra agglutinin and, after treatment with neuraminidase, bound to PNA. Taken together these data suggest the presence of O-linked oligosaccharides on phosphodiester α-GlcNAcase.

Although, as pointed out in Table II, the lectin blot experiments cannot provide complete oligosaccharide structure determination, the specificity of the lectins unequivocally demonstrated the presence of sialic acid and galactose on the enzyme. Since phosphodiester α-GlcNAcase has sialic acid α2,3Gal on N- and possibly O-linked oligosaccharides and...
sialic acid α2,6GalNAc residues on O-linked oligosaccharides, it presumably has been acted upon by galactosyl and sialyltransferases which are localized to the trans-Golgi and TGN.

The acquisition of terminal glycosylation by residents of the cis- and medial-Golgi has been reported. GIMP, a cis-Golgi marker (14), MG-160 (15), and α-mannosidase II (16), both medial-Golgi markers, all have sialic acid residues on their N-linked carbohydrates. However, all resident Golgi glycoproteins do not undergo terminal glycosylation. Mannosidase IA, concentrated in the medial-Golgi, and p58, a cis-Golgi marker, lack terminal processing by sialyltransferase or galactosyltransferase (17, 18). Furthermore, the glycosylation of Golgi membrane glycoproteins may vary from cell type to cell type. α-Mannosidase II, a medial-Golgi processing enzyme, contains complex N-linked oligosaccharides and O-linked oligosaccharides in 3T3 cells but only N-linked oligosaccharides in HeLa cells (16). Assuming that phosphodiester α-GlcNAcase is localized to the cis-medial-Golgi, it is formally possible that the terminal processing of its oligosaccharides occurs as the newly synthesized processing enzymes are traversing the Golgi. Alternatively, a percentage of phosphodiester α-GlcNAcase may cycle through the Golgi stacks and be retrieved in the trans-Golgi or TGN, while maintaining residence in the cis-medial-Golgi.

Immunofluorescence studies using a monoclonal antibody directed against phosphodiester α-GlcNAcase have allowed us to analyze the intracellular location of this enzyme. In Vero cells, phosphodiester α-GlcNAcase is concentrated in a perinuclear “Golgi-like” structure. A very similar staining pattern was observed with α-mannosidase II, a medial-Golgi marker. In the presence of BFA, phosphodiester α-GlcNAcase disperses into an ER pattern, consistent with the finding that BFA causes the Golgi cisternae to disperse into the ER pattern (12, 19). In contrast WGA staining is less dispersed in the presence of BFA, and the staining is clearly distinct from that obtained for phosphodiester α-GlcNAcase. Recently, others have reported the formation of a hybrid network of TGN and early endosomes in cells treated with BFA (20–22), a network distinguishable from that formed by Golgi stack proteins. WGA stains heavily sialylated glycoproteins of the trans-Golgi and TGN and most likely accumulates in this hybrid network of TGN and early endosomes after treatment with BFA. In contrast, we believe phosphodiester α-GlcNAcase is most likely a resident of the Golgi stacks because, after treatment with BFA, it distributes to an “ER-like” compartment, distinct from the hybrid network of TGN and endosomes. Interestingly, Sampath et al. (23) found that cells treated with 0.1 μg/ml BFA showed an 80% decrease in their phosphorylated oligosaccharides (derived from lysosomal enzymes), and the majority of the phosphorylated species contained phosphodiesterases, i.e. were not recovered. In an earlier study, Radons et al. (24) found that the phosphorylation of oligosaccharides on the lysosomal enzyme cathepsin D was diminished in BFA-treated cells and that the uncovering of the phosphate groups was abolished. Both studies indicate that phosphodiester α-GlcNAcase is relatively inactive on oligosaccharides of lysosomal enzymes in BFA-treated cells, and both groups suggest that phosphodiester α-GlcNAcase is, therefore, in a compartment distal to the BFA block. However, since our data show that the enzyme is dispersed into the ER in the presence of BFA, its low activity may be caused by dilation or unfavorable conditions in that compartment. To identify the intracellular localization of phosphodiester α-GlcNAcase precisely it will be necessary to perform immunoelectron microscopy using antibodies directed against phosphodiester α-GlcNAcase.

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