Human Mannose 6-Phosphate-uncovering Enzyme Is Synthesized as a Proenzyme That Is Activated by the Endoprotease Furin*

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EXPERIMENTAL PROCEDURES

Materials

UDP-[3H]GlcNAc was purchased from PerkinElmer Life Sciences. 4-Methylumbelliferyl β-D-glucuronide, 4-methylumbelliferyl N-acetyl-β-D-glucosaminide, Man-6-P, and Escherichia coli alkaline phosphatase were obtained from Sigma. The bovine Man-6-P/IGF-II receptor affinity column (2.0 mg/ml) was provided by Walter Gregory (Washington University, St. Louis, MO). Furin and PNGase F were purchased from New England Biolabs Inc. Furin inhibitor I (decanoyl-Arg-Val-Lys-Arg-chloromethyl ketone) was purchased from Calbiochem. The mouse HPC4 monoclonal antibody was obtained from Dr. Charles Esmon (Oklahoma Medical Research Foundation) (7). Rabbit anti-UCE propeptide serum (NZW-HD-9B) was prepared by immunization with a synthetic peptide corresponding to the 24-amino acid propeptide of human UCE, and rabbit anti-rh-UCE (NZW-HD-1A) serum was prepared by immunization with rh-UCE lacking the transmembrane and cytoplasmic tail domains.

Cell Lines

Mouse L cells and L cells expressing wild-type (WT 1–38) or mutant (Y188A) human UCE were obtained from Rosalind Kornfeld (Washington University) and grown as described (2). LoVo human colon adenocarcinoma cells (CCL-229) were purchased from American Type Culture Collection and grown in Kainh’s modification of Ham’s F-12 medium with 10% fetal bovine serum. CHO-K1 cells and SF² cells were obtained from American Type Culture Collection and from Protein Sciences Corporation, respectively.

Enzyme Assays

UCE was assayed as previously described using [3H]GlcNAc-α-Me-P-Man as substrate (8). One unit of activity is defined as 1 nmol of

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**Fig. 1. Human UCE sequence with the putative furin cleavage site.** The upper diagram shows the sequence of the signal peptide, propeptide, and the beginning of the mature portion of human UCE. The putative furin cleavage site is shown. The lower diagram shows how the AcNPV signal peptide is directly linked to the mature portion of human UCE in the insect cell expression vector. Bves, baculovirus expression vector system.

[^1]GlcNAc released per h. β-Glucuronidase and β-hexosaminidase activities were determined fluorometrically. The reactions were carried out for 1 h at 37 °C in a 100-μl volume containing 5 mM 4-methylumbelliferyl β-D-glucuronide or 4-methylumbelliferyl N-acetyl-β-D-glucosaminide in 0.1 M sodium acetate (pH 5.0). The reactions were stopped by addition of 800 μl of 0.1 M sodium carbonate solution, and the fluorescence was determined in a Turner digital fluorometer.

**Plasmid Construction for Expression of rh-UCE in Mammalian Cells**

The molecular cloning and expression of wild-type human UCE has been described previously (3). This plasmid, designated as pTriplEx/UCE, was used as the starting material for the construction of recombinant soluble epitope-tagged UCE.

Several intermediate plasmids (pKB3, pKB4, and pKB5) were constructed to provide the appropriate DNA fragments with the necessary restriction sites. Plasmid pKB3 was constructed by inserting a pair of complementary oligonucleotide linkers (CANF381, 5'-CCGGGAGGCTCCATGAGGAGGAGAGGCCGGCCCCTGAGGT-3'; and CANF382, 5'-GATCTGGAGTGTTCCCGCCCGAGAGGGTACCTGCGGACG-3') into pUC19 (New England Biolabs Inc.) between the XmaI and XbaI restriction sites. Plasmid pKB3 was constructed by ligating the FesI-BsaI fragment of pTriplEx/UCE (amino acids 93–437 of human UCE), the XbaI-B rescued and subsequently amplified in 96-well plates at various MSX concentrations (100–500 μM). The clones with simplified expression were initially identified by a sandwich enzyme-linked immunosorbent assay using HPC4 as the capture antibody, a rabbit anti-rh-UCE polyclonal primary antibody, and horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies. These amplified UCE clones were also assayed for UCE activity.

A rapidly growing MSX-amplified UCE clone with high UCE expression (designated as UCE108) was identified from a 96-well plate containing 300 μM MSX via enzyme-linked immunosorbent assay and was derived from the unamplified parent clone MB7. Clone UCE108 was expanded and used for the production of rh-UCE.

**Plasmid Construction for Expression of rh-UCE in Insect Cells**

Plasmid pKB6 was used to construct a plasmid encoding soluble epitope-tagged human UCE in an insect expression system (performed at Protein Sciences, Inc.). PCR was performed using pKB6 and gene-specific primers O-1987/O-1989 to generate the N terminus of the mature human UCE region and the C-terminal fragment of the signal sequence from Autographa californica nuclear polyhedrosis virus (AcNPV). A parallel PCR was performed to generate the polyhedrin promoter and the AcNPV signal sequence. A third PCR was performed to fuse the N terminus of mature human UCE with the AcNPV promoter/signal sequence containing a 5′-EcoRV restriction site and a 3′-BglII site via overlap extension. The resultant PCR product was digested with EcoRV and BglII and ligated into an intermediate plasmid. This intermediate plasmid was then digested with KpnI and XbaI and ligating this fragment into the linearized intermediate plasmid to complete the transfer plasmid designated as pAcD1062. The DNA sequence was verified by automated fluorescence DNA sequencing, and pAcD1062 was used to generate the baculovirus transfer vector.

To generate the baculovirus vector via homologous recombination, the transfer plasmid pAcD1062 was cotransfected with linearized AcNPV baculovirus genomic DNA for 3 days. The cotransfected cells were subsequently harvested by centrifugation, and the supernatants were used to grow isolated plaques on plates containing Sf9 insect cells. Several plaques with the clear (versus cloudy) plaque phenotype were identified from this process and used as the viral stocks.

**Expression of Recombinant Soluble Human UCE in Insect Cells**

The expression of rh-UCE in insect cells was performed at Protein Sciences, Inc. A passage 1 viral stock of recombinant baculovirus was prepared by adding the appropriate plaque to a 5-mi culture of Sf9
insect cells in medium containing 5% FBS for 5 days at 28 °C. The infected cells were subsequently harvested by centrifugation, and the supernatant was used to inoculate larger cultures. One milliliter of the supernatant was used to inoculate larger cultures. One milliliter of the supernatant was used to inoculate larger cultures. One milliliter of the supernatant was used to inoculate larger cultures.

### Purification of rh-UCE from CHO Cell-conditioned Medium—Insect Expression Systems

**Electrophoresis and Immunoblotting**

Two-hundred nanograms of each PNGase F-digested rh-UCE sample was subjected to SDS-PAGE using 12% polyacrylamide gels (Invitrogen). rh-UCE was transferred to nitrocellulose using a Bio-Rad semidry transfer system. The membrane was subsequently blocked with 5% (w/v) nonfat dry milk in 150 mM NaCl, 50 mM Tris-HCl (pH 7.5), and 1 mM CaCl₂ (blocking buffer) for 1 h at room temperature with rocking. The blot was then incubated with the mouse HPC4 monoclonal primary antibody (1 μg/ml) in blocking buffer for 30 min at room temperature with rocking. The blot was then incubated with the mouse HPC4 monoclonal primary antibody (1 μg/ml) in blocking buffer for 30 min at room temperature with rocking.

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**TABLE I**

| Sample          | Volume | Protein conc | Total protein | Activity | Total activity | Specific activity | Yield |
|-----------------|--------|--------------|---------------|----------|---------------|------------------|-------|
| CHO cells       |        |              |               |          |               |                  |       |
| Medium (3× conc)| 1400   | 17.4         | 24,360        | 8.7 × 10⁵ | 12.2 × 10⁶    | 501              | 100   |
| HPC4 eluate     | 1      | 44.6         | 44.6          | 11.1 × 10⁵| 11.1 × 10⁵    | 2.5 × 10⁵        | 91    |
| SF² cells       |        |              |               |          |               |                  |       |
| Medium          | 100    | 15.2         | 1520          | 6 × 10⁵  | 6 × 10⁵       | 395              | 100   |
| HPC4 eluate     | 1      | 0.52         | 0.52          | 2.85 × 10⁵| 2.85 × 10⁵    | 5.5 × 10⁵        | 48    |

**TABLE II**

| Cycle | Amino acid | Unprocessed | Processed |
|-------|------------|-------------|-----------|
| 1     | Leu, Asp   | 69          | 31        |
| 2     | Asp        | 100         | ND        |
| 3     | Ser, Thr   | 60          | 40        |
| 4     | Gly        | 100         | ND        |
| 5     | Ala, Val   | 68          | 32        |
| 6     | Ser        | 100         | ND        |
| 7     | Arg        | 100         | ND        |
| 8     | Asp, Gly   | 64          | 36        |
| 9     | Asp, Asn   | 55          | 45        |
| 10    | Asp        | 100         | ND        |
| 11    | Leu, Glu   | 71          | 29        |

**Fig. 2. Distinction of pro-UCE and mature UCE by dot-blot analysis.** Three identical nitrocellulose dot blots were prepared using 200 ng of rh-UCE derived from either insect or CHO cells. A rh-UCE molecule engineered to contain an improved furin cleavage site (DB-1 rh-UCE) was also expressed in CHO cells and spotted onto each membrane. A buffer control (150 mM NaCl, 50 mM Tris (pH 7.2), and 5 mM EDTA) was also included in each dot blot. The dot blots were subsequently treated as described for the immunoblots under “Experimental Procedures,” except that the blot in A was incubated with rabbit anti-UCE propeptide serum (NZW-HD-9B), the blot in B was incubated with rabbit preimmune serum, and the blot in C was incubated with protein A-purified rabbit anti-rh-UCE (whole molecule) monoclonal antibody (NZW-HD-1A, diluted 1:5000 in blocking buffer). The detection antibody was horseradish peroxidase-conjugated donkey anti-rabbit antibody (diluted 1:10,000 in blocking buffer; Amersham Biosciences).
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RESULTS

Pro-UCE Is Inactive—As part of a project to prepare soluble forms of UCE, the cDNA encoding human UCE was mutated to introduce a stop codon just proximal to the transmembrane domain and then expressed in CHO and SF21 insect cells. The expression vector for the insect cells was further modified to include an insect viral signal peptide in place of the UCE signal peptide and a deletion of the propeptide (Fig. 1). When rh-UCE secreted from the two cell types was purified and assayed, it was noted that the specific activity of the CHO cell-produced enzyme was only 45% that of the insect cell-secreted enzyme (Table I). Determination of the N-terminal sequence of rh-UCE produced by the CHO cells revealed that only 35% of the enzyme was the mature form, whereas 65% remained as the proenzyme (Table II). To confirm the presence of the propeptide in a portion of CHO cell-produced rh-UCE, a rabbit antibody was generated to the propeptide sequence. As shown in Fig. 2, this antiserum reacted with CHO cell-produced rh-UCE, but not with rh-UCE secreted by the insect cells. The enzymes from both sources reacted with rabbit antiserum prepared against full-length rh-UCE as expected.

The fact that only 35% of rh-UCE secreted by the CHO cells was the mature form and that this material had only 45% the specific activity of insect cell-derived rh-UCE, which was completely the mature form, suggested that the pro-form of rh-UCE may have little or no enzyme activity. Evidence that this

FIG. 3. Effect of furin on the enzyme activity of CHO cell-produced rh-UCE. Aliquots (20 µg) of purified CHO cell-derived (■) and insect cell-derived (○) rh-UCE were incubated either with 20 units of furin (■) or without furin (○) at 30 °C in a 75-µl assay containing 100 mM Hepes (pH 7.5), 0.5% Triton X-100, and 1 mM 2-mercaptoethanol for the times indicated. Two microliters was removed from each sample and diluted 1:1000 to 1:1600 with 50 mM Tris-HCl (pH 6.7) and 0.5% Triton X-100 and assayed for UCE activity using the [3H]GlcNAc-α-Me-P-Man substrate. The UCE activity was calculated for each sample and normalized to the corresponding samples that lacked furin. Data are plotted as the percent increase in UCE activity as a function of incubation time.

peroxidase-conjugated sheep anti-mouse secondary antibody (Amersham Biosciences) diluted 1:10,000 in blocking buffer for 30 min at room temperature with rocking. The blot was washed as before and incubated with 150 mM NaCl, 50 mM Tris-HCl (pH 7.5), and 1 mM CaCl2 for 15 min. rh-UCE was visualized by incubating the blot with 1 ml of ECL chemiluminescent substrate (Amersham Biosciences) for 1 min, wrapped in plastic wrap, and exposed on BioMax x-ray film (Eastman Kodak Co.).

N-terminal Sequencing of rh-UCE

Three micrograms each of purified rh-UCE from insect and CHO cells were subjected to SDS-PAGE (12% polyacrylamide gel) and transferred to polyvinylidene difluoride membrane (Amersham Biosciences) using the Bio-Rad semidry transfer system. The protein bands were wrapped in plastic wrap, and exposed on BioMax x-ray film (Eastman Kodak Co.).

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Man-6-P/IGF-II Receptor Affinity Chromatography

β-Glucoronidase and β-hexosaminidase present in cell secretions were fractionated on a Man-6-P/IGF-II receptor affinity column essentially as previously described (9). Briefly, media from LoVo and L cell cultures were collected and applied to the receptor affinity column (1 × 1 cm). The column was washed with 5 ml of phosphate-buffered saline (the flow-through fraction), then with 5 ml of phosphate-buffered saline containing 5 mM Glc-6-P (the retarded fraction), and finally with 5 ml of phosphate-buffered saline containing 5 mM Man-6-P (the bound/eluted fraction). Aliquots of the three fractions were assayed for β-glucoroni-
dase and β-hexosaminidase activities. To demonstrate the effect of UCE on the secretion hydrodases, 1-ml aliquots of the media were treated with 10 units of rh-UCE for 4 h at 37 °C and then applied to the affinity column and fractionated as described above. Similarly, to differentiate between hydrodases with phosphomonoesters versus multiple phosphodiesterases, 1-ml aliquots of the media were treated with 50 units of E. coli alkaline phosphatase at pH 8.0 for 12 h at 37 °C. The reactions were adjusted to pH 7.4 and then subjected to fractionation on the receptor column.

FIG. 4. Pro-UCE is cleaved by furin. Twenty micrograms each of baculovirus expression vector system and CHO cell-produced rh-UCE were treated with 20 units of furin at 30 °C, and aliquots were removed after 0, 6, 12, and 24 h. The baculovirus expression vector system and CHO cell-produced enzymes without furin treatment were used as negative controls. The UCE samples were then deglycosylated by incubation with PNGase F, and 200 ng of each sample was subjected to SDS-PAGE, followed by Western blotting using the mouse HPC4 primary antibody and horseradish-conjugated sheep anti-mouse secondary antibody.

| Addition Mouse L cells | WT 1–38 cells | LoVo cells |
|------------------------|---------------|------------|
| None                   | 0.27          | 2.92       | 0.02      |
| 1 unit furin           | 0.26          | 2.97       | 0.27      |
| 2 units furin          | 0.26          | 2.97       | 0.34      |
| 4 units furin          |               |            |           |
is indeed the case was obtained by treating rh-UCE produced by the CHO cells with furin, which was predicted to cleave the propeptide. As shown in Fig. 3, furin treatment resulted in a marked increase in enzyme activity to the level observed with the enzyme expressed by insect cells. CHO cell-derived rh-UCE incubated in the absence of furin exhibited no change in its activity, and furin had no effect on the activity of insect cell-derived rh-UCE. Examination of CHO cell-derived rh-UCE by Western blotting using monoclonal antibody HPC4 following deglycosylation with PNGase F revealed two bands corresponding to the pro-form and mature form of the enzyme (Fig. 4). Treatment of this material with furin caused the conversion of the pro-form to the mature form. This did not occur in the absence of furin. Furthermore, furin had no effect on insect cell-derived rh-UCE, demonstrating that furin cleaves only the propeptide of UCE.

**Furin Is Required for UCE Activity in LoVo Cell Extracts**

The experiments with CHO cell-derived rh-UCE clearly show that furin is capable of cleaving the propeptide of UCE. However, these results do not establish that furin is the endoprotease that acts on UCE in cells. This is an issue because furin is a member of a large family of proprotein convertases (6). To test whether furin is involved in the activation of UCE in intact cells, we turned to LoVo cells, derived from a lymph node

**FIG. 5. Furin activates UCE in LoVo cell extracts.** LoVo cells and WT 1–38 mouse L cells were grown to 90% confluence in 24-well plates. After removal of the media, 120 µl of 100 mM Hepes (pH 7.5) containing 1% Triton X-100, 1 mM CaCl₂, 1 mM 2-mercaptoethanol, and 2 units of furin was added, and the lysed cell extracts were incubated at 25 °C. At the indicated times, the samples were assayed for UCE activity. Data are plotted as a percent of the zero time value. The starting LoVo cell extract contained 0.7 units of UCE activity/mg of protein, whereas the WT 1–38 cell extract contained 70 units of UCE activity/mg of protein. ○, LoVo cells; ▲, WT 1–38 cells.

**FIG. 6. Furin inhibitor I inhibits rh-UCE processing in CHO cells.** A CHO cell line that stably expresses rh-UCE was incubated with furin inhibitor I at 0, 5, 10, or 20 µM for 72 h at 37 °C. Secreted rh-UCE was subsequently purified via HPC4-Sepharose. Five micrograms of rh-UCE purified from the 20 µM furin inhibitor culture was incubated with 10 units of furin for 24 h at 30 °C. All samples were then assayed for UCE activity. Each purified rh-UCE sample (3 µg) was digested with PNGase F, and 150 ng of each sample was subjected to SDS-PAGE and Western blotting using the HPC4 primary antibody. BVES, baculovirus expression vector system.
metastasis of a human colon adenocarcinoma (10). These cells are known to have a total deficiency of furin activity due to mutations in the furin gene (10). Assay of LoVo cell extracts for UCE activity revealed that these cells had less than 10% of the activity of mouse L cells (Table III). When LoVo cell extracts were treated with different concentrations of furin for 4 h, the UCE activity increased up to 17-fold to the level observed in the mouse L cell extract (Table III). Furin treatment had no effect on the UCE activity of the mouse L cell extract or an extract of mouse L cells (the WT 1–38 line) that express high levels of UCE (Table III). The activation of UCE in the LoVo cell extract by furin was time-dependent, as shown in Fig. 5. These results demonstrate that the LoVo cells contain UCE in an inactive form (presumably the pro-form) that can be activated by furin. Because these cells have a selective deficit in furin activity, we conclude that UCE is specifically activated by furin.

A Furin Inhibitor Blocks Processing of Pro-UCE—We next tested the effect of the furin inhibitor decanoyl-Arg-Val-Lys-Arg-chloromethyl ketone (11) on the maturation of pro-UCE. For this experiment, the CHO cells expressing rh-UCE were incubated with various concentrations of the inhibitor for 72 h, and then secreted rh-UCE was purified and analyzed by SDS-PAGE. As shown in Fig. 6, the inhibitor caused a concentration-dependent shift in secreted rh-UCE from the mature form to the pro-form. This was associated with a decrease in the specific activity of the enzyme. When UCE was synthesized in the presence of 20 μM furin inhibitor I was treated with furin, all of the pro-form was converted to the mature enzyme along with the restoration of the enzyme activity (Fig. 6, sixth lane). These data provide additional evidence that UCE maturation is mediated by furin.

UCE Cleaves UDP-GlcNAc as Well as GlcNAc-α-P-Man—In considering why it may be advantageous to activate pro-UCE only after it reaches the TGN, we noted the reports that partially purified detergent-solubilized preparations of UCE cleave GlcNAc from UDP-GlcNAc (12–14). Because many Golgi N-acetylgalactosaminyltransferases utilize UDP-GlcNAc as their sugar donor, it might be detrimental if UCE were active as it passed through the Golgi stack. To confirm this observation with a membrane-bound form of the enzyme, we assayed intact mouse cells expressing human UCE(Y488A) on their surface. This form of UCE contains a mutation in the 488YHPL internalization signal present in the cytoplasmic tail; and consequently, it accumulates on the cell surface (2). When monolayer cultures of these cells were incubated with medium containing either UDP-[3H]GlcNAc or [3H]GlcNAc-P-Man and the release of [3H]GlcNAc was determined, it was evident that surface UCE acted equally on both substrates (Fig. 7). The non-transfected mouse L cells did not hydrolyze detectable amounts of UDP-[3H]GlcNAc under these assay conditions. This finding
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confirms that UCE is capable of hydrolyzing UDP-GlcNAc as well as GlcNAc-α-P-Man.

LoVo Cells Secrete Acid Hydrolases with Phosphomannose Diesters—Because LoVo cells have very little UCE activity, we would expect the cells to secrete acid hydrolases that contain phosphomannose diesters rather than the monoesters found on acid hydrolases secreted by cells that have sufficient UCE activity. To analyze this, culture medium from LoVo cells was passed over a Man-6-P/IGF-II receptor affinity column, and the flow-through, retarded, and Man-6-P-eluted fractions were assayed for β-glucuronidase and β-hexosaminidase activities. Most of the enzyme activity was in the flow-through fraction, with only 10–20% in the Man-6-P-eluted fraction (Fig. 8A). The material retained could represent enzyme with phosphomonoester(s) or multiple phosphodiester(s) because the latter have been shown to be capable of binding to Man-6-P/IGF-II receptor affinity columns (15). To distinguish between these possibilities, an aliquot of the medium was treated with E. coli alkaline phosphatase, which can cleave only the phosphate from phosphomonoesters, and reapplied to the receptor column. This treatment decreased the binding of the two acid hydrolases by ~50% (Fig. 8B). Therefore, only 5–10% of the acid hydrolases in the medium contained phosphomonoesters. When the medium was treated with rh-UCE prior to passage over the receptor affinity column, 65% of the β-glucuronidase and almost 90% of the β-hexosaminidase bound to the column and required Man-6-P for elution (Fig. 8C). This binding was abolished when the medium was subsequently treated with alkaline phosphatase (Fig. 8D). These data show that the acid hydrolases secreted by the LoVo cells contained mostly phosphomannose diesters. In contrast to these results, the majority of the β-glucuronidase and β-hexosaminidase present in the medium of mouse L cells bound to the receptor affinity column, and this binding was mostly abolished by alkaline phosphatase treatment (Fig. 9, A and B). Furthermore, incubation with rh-UCE did not enhance binding to the affinity column (Fig. 9C), indicating that cellular UCE had efficiently converted the diesters to monoesters in the L cells. Consistent with this result, treatment with rh-UCE and alkaline phosphatase abolished receptor binding (Fig. 9D).

DISCUSSION

The findings presented in this study establish that UCE is synthesized as a zymogen that is activated by furin. The strongest evidence that furin serves as the endoprotease that cleaves the propeptide of UCE in vivo comes from the studies with LoVo cells. These cells have been documented to be deficient in UCE activity due to a frameshift mutation in one allele and a point mutation within the homo B domain in the other allele of the furin gene (10). Furin is a member of the subtilisin-like protease family, which includes seven members to date (6). It is known to catalyze the maturation of a diverse group of proprotein substrates by cleaving most efficiently and specifically at the C-terminal side of an Arg(P<sub>4</sub>-X-Lys/Arg-P<sub>4</sub>) sequence. The arginine residues at positions −1 and −4 are essential, and basic residues at positions −2 and −6 facilitate efficient cleavage (16–18). The RARLP|D cleavage site on pro-UCE shows the RXRX|D arrangement typical for furin cleavage, and the additional arginine at position −6 probably increases the efficiency of furin-mediated cleavage. Proline at position −2 has no negative effect on furin cleavage (19). A similar RLP|E sequence is present on β-secretase or BACE (beta-site APP-cleaving enzyme), which cleaves the amyloid precursor protein to release the amyloid peptide, which is the main constituent of the amyloid plaques in the brains of Alzheimer’s disease patients (20). This propeptide has been convincingly shown to be processed by furin.

There is also strong evidence that furin processes Pseudomonas exotoxin A that has a proline at position −2 (21, 22).

It is of note that the LoVo cell extracts exhibited a low level of UCE activity and that 5–10% of the secreted acid hydrolases contained phosphomonoesters. There are at least three possible explanations for this. First, the pro-form of UCE may have some activity. At this time, we do not have a homogeneous preparation of the pro-form of UCE to test for possible activity. Second, another proconvertase present in the LoVo cells may have some activity toward pro-UCE. A number of studies have reported that LoVo cells efficiently process a few of the propeptides that furin is known to act upon, indicating that LoVo cells express other proconvertases (23–27). However, all these substrates have the sequence Arg-X-Lys/Arg-X or Lys-X-Arg at their cleavage site. In contrast, LoVo cells act very inefficiently on Pseudomonas exotoxin and Shiga toxin that have the cleavage sequence Arg-X-X-Arg, which is present in UCE (28, 29). Despite this, we cannot exclude the possibility that one of the proconvertases expressed in the LoVo cells is activating a small proportion of UCE. Finally, it is possible that another enzyme has a slight ability to cleave GlcNAc from GlcNAc-P-Man diesters. Even if this is the case, it is clear that UCE accounts for the vast majority of the uncovering activity.

Although the propeptides of many proteins are necessary for correct folding to form an active protein, this appears not to be the case for UCE. As shown in Table I, a construct expressing a form of UCE that lacks the propiece folds into a fully active enzyme, at least in insect cells grown at 25 °C. This indicates that the primary function of the propiece is to inhibit UCE activity until it arrives at the TGN, where it encounters furin. Why is it important to avoid having an active form of UCE in the Golgi stack? One possibility is to prevent the hydrolysis of UDP-GlcNAc in the lumen of the Golgi cisternae. UDP-GlcNAc is the nucleotide sugar donor for the many N-acetylglucosaminyltransferases that are localized in the Golgi stack. Our studies with the cell-surface form of UCE (Fig. 7) show that this enzyme acts on UDP-GlcNAc about as well as it cleaves GlcNAc-P-Man. Similar findings have been reported for the solubilized form of the enzyme (12–14). Therefore, if UCE were active as it moved through the Golgi on its way to the TGN (or if it resided in the Golgi), it might interfere with the assembly of complex-type N-linked glycans and O-linked glycans by depriving the N-acetylglucosaminyltransferases of their donor substrate, UDP-GlcNAc. By activating UCE only after it arrives in the TGN, where it is localized, this potential problem is avoided.

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