Endoplasmic Reticulum Glucosidase II Is Composed of a Catalytic Subunit, Conserved from Yeast to Mammals, and a Tightly Bound Noncatalytic HDEL-containing Subunit*

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Trimming of glucose from N-linked core glycans on newly synthesized glycoproteins occurs sequentially through the action of glucosidases I and II in the endoplasmic reticulum (ER). We isolated enzymatically active glucosidase II from rat liver and found that, in contrast with previous reports, it contains two subunits (α and β). Sequence analysis of peptides derived from them allowed us to identify their corresponding human cDNA sequences. The sequence of the α subunit predicted a soluble protein (104 kDa) devoid of known signals for residence in the ER. It showed homology with several other glucosidases but not with glucosidase I. Among the homologues, we identified a Saccharomyces cerevisiae gene, which we showed by gene disruption experiments to be the functional catalytic subunit of glucosidase II. The disrupted yeast strains had no detectable growth defect. The sequence of the β subunit (58 kDa) showed no sequence homology with other known proteins. It encoded a soluble protein rich in glutamic and aspartic acid with a putative ER retention signal (HDEL) at the C terminus. This suggested that the β subunit is responsible for the ER localization of the enzyme.

The oligosaccharide that initiates N-glycosylation of proteins, Glc₃Man₉GlcNAc₂, is synthesized in the endoplasmic reticulum (ER), linked by a pyrophosphate group to dolichol (1). The oligosaccharide portion is transferred en bloc to the appropriate consensus sequences (Asn-X-Ser/Thr) of nascent polypeptide chains. Immediately after transfer, the trimming process starts; glucosidase I, a membrane-bound enzyme removes the terminal α-1,2-linked Glc residues. Glucosidase II, a soluble or peripherally attached luminal enzyme, then removes the following two α-1,3-linked Glc units (1–3). The resulting glucose-free, high mannose sugar chains can be reglucosylated in the ER by the UDP-Glc:glycoprotein glucosyltransferase, a luminal enzyme that utilizes unfolded or misfolded glycoproteins as substrates (4). The added Glc residues are believed to be removed by glucosidase II.

The de- and reglucosylation events in the ER are thought to be part of a glycoprotein-specific folding and quality control machinery. According to a model proposed by Hammond and Helenius (5), the monoglucosylated carbohydrate units (generated either by initial trimming of the core glycans or by reglucosylation) mediate interaction of newly synthesized glycoproteins with a resident ER chaperone called calnexin. Calnexin, a membrane protein, and its soluble homologue calreticulin are known to behave as lectins, binding monoglucosylated newly synthesized glycoproteins (6–10). They retain glycoproteins in the ER while these are undergoing folding and, in some cases, oligomeric assembly. They also promote folding and oligomerization (11, 12). Once glycoproteins have adopted their mature, fully folded conformation, they are no longer recognized by the glucosyltransferase and are no longer reglucosylated (13). When glucosidase II removes the remaining Glc residues from such glycoproteins, they move out of the cycle and continue their maturation along the secretory pathway.

In this chaperone cycle, glucosidase II has two separate functions. By removing the middle glucose, it allows binding of glycoproteins to calnexin and calreticulin, and by removing the innermost glucose, it releases the bound glycans from the lectins and prevents rebinding.

Several reports have been published on the isolation and characterization of glucosidase II from different sources (14–18). Some of them (14, 17) identified it from animal tissues as a 60–70-kDa protein. In other cases (15–17), it was characterized as a 100–120-kDa polypeptide. No sequence data has been reported for glucosidase II from any source.

To get further insight into the properties of this enzyme, we wanted to develop a reliable and reproducible purification procedure from rat liver microsomes that would allow us to clone the cDNA coding for it and to carry out structural studies. On doing so, we obtained a purified enzyme that was different from that described in previous reports. Instead of a single polypeptide chain, we found that the enzyme was a heterodimer of two distinct subunits. Sequence analysis of peptides derived from each subunit allowed us to identify their respective human cDNAs. We also identified a Saccharomyces cerevisiae gene very similar to the larger subunit and showed it to be the functional glucosidase II homologue in yeast. Together with the sequence homology with known glucosidases, this indicated that the large subunit was the catalytic component of glucosidase II. The smaller subunit is likely to mediate ER retention and retrieval of the enzyme.

EXPERIMENTAL PROCEDURES

Materials—DEAE-Sephalose, concanavalin A-Sepharose, Mono Q, and Superdex columns were from Pharmacia Biotech Inc., ceramic hydroxylapatite was from Bio-Rad, and Poros HP 10 phenyl column was...
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from Perseptive Biosystems (Cambridge, MA). All chemicals were from Sigma Chemical Corp. (St. Louis, MO).

Assays—Protein concentration was determined by the method of Lowry (19). Glucosidase II activity was measured by employing p-nitrophenyl-α-D-glucopyranoside as substrate. Incubations were carried out in 20 mM HEPES buffer, pH 7.5, 5 mM substrate, in a total volume of 50 μl. After the indicated time at 37 °C (typically 5–30 min) reactions were stopped by adding 50 μl of 2 M Tris base, and absorbance at 455 nm was determined. [14C]Glucose–Glc-Man α,Man, GlcNAc, [13C]glucose–Glc-Man,GlcNAc, and [14C]glucose–Glc-Man,GlcNAc, and substrate were added to a final concentration of 0.2% SDS and 20 mM DTT. The gel was treated as described previously (20, 21).

Purification of Rat Liver Glucosidase II—All the following steps were carried out on ice or at 4 °C in a cold room. Protease inhibitors were included in all of the buffers for the preparation of the microsomes and the extraction of glucosidase II. Inhibitors used were pepstatin, antipain, epoxomicin, leupeptin, and aprotinin.

2. MgCl₂ were added to 1 mM and loaded onto a column of concanavalin A-Sepharose in 20 mM HEPES buffer, pH 7.5, 5 mM substrate, in a total volume of 50 μl. Elution was performed by a linear gradient of 30 ml from 0 to 0.5 M NaCl in buffer D at a flow rate of 1 ml/min. 0.5-ml fractions were collected and assayed for glucosidase II activity.

Gel Filtration—Activity eluted from the concanavalin A-Sepharose was diluted with more buffer D and run on an SPS-50E gels and transferred to Immobilon-P membranes. For internal sequence analysis, purified glucosidase II was run on SDS-PAGE, gels were Coomassie-stained, and the individual bands were digested in situ with trypsin. Peptides were eluted, alkylated, and separated by reverse phase high pressure liquid chromatography as described in Ref. 25.

Disruption of the α Subunit in S. cerevisiae—For disruption of the α subunit gene in yeast, a polymerase chain reaction fragment was generated that included nucleotides 226–270 (5′-CTC ACG CCA CTT GAG AGC TAT ACA TGA ATG TGG-3′) and nucleotides 3184–3225 (5′-GTT ATT TTT TGA GGG AAA AAA CCG AAG TAG TAT CCT TAC-3′) of gene Z5S098 at the 5′- and 3′-ends, respectively, of the URA3 gene (26). Strains NY914 (MATa, leu2-3,112, ura3-52, his3Δ200) and NY920 (MATa, leu2-3,112, ura3-52, his3Δ200, ade2-1A200) were transformed with the polymerase chain reaction fragment according to a standard protocol (27). Uracil prototrophs were analyzed for disruption of the α subunit by a polymerase chain reaction approach, by using either two primers that were proximal to those used for the disruption (positions 208–225, 5′-GCC GCA CCA CAT ATT G, for the sense primer; positions 3243–3266, 5′-ACG GAT ATT TTT ACG TTT ACT TTG, for the antisense primer) or the α subunit primer 208–225 and a URA3 gene-specific antisense primer.

Western Blot Analysis—Yeast cells were grown to an optical density of 0.5–0.7 at 600 nm and disrupted by vortexing in the presence of glass beads followed by boiling. To prevent glucose trimming or protein degradation during and after lysis, 1 mM phenylmethylsulfonyl fluoride (Boehringer Mannheim); 10 μM each chymostatin, leupeptin, antipain, and pepstatin; and 5 mM castanospermine (Sigma) were added to the lysate buffer (0.1% SDS, 100 mM Tris-HCl, pH 7.5). For Endo H treatment, 5.25 sodium acetate at 200 μM final concentration and 5 × 10⁻⁴ units of Endo H (Boehringer Mannheim) were added to the cell lysates, and samples were incubated for 14 h at 37 °C. Samples were separated by 15% SDS-PAGE, electrotransferred to nitrocellulose filters, and blotted for protein disulﬁde isomerase (PDI) (28) according to standard protocols.

Radio labeling and Immunoprecipitation of ER-retained carboxypeptidase Y (CPY)—Yeast cells were grown overnight to an early logarithmic phase in SC medium supplemented with the appropriate amino acids, peptide, and resuspended in a small volume of medium (1.3 optical density units/200 μl of medium). After a preincubation at 37 °C in the presence of 10 mM DTT, 100 μCi of [35S]methionine and [35S]cysteine (L-[35S]S) in Vitro Cell Labeling Mix; Amersham) was added, and the incubation continued for another 30 min. Protein synthesis was terminated by washing the cells in 2 volumes of ice-cold phosphate-buffered saline containing 20 mM sodium azide, and the cells were lysed as described for the Western blot analysis. The samples were 10-fold with 1% Triton X-100 in phosphate-buffered saline and the protease inhibitors described above, and CPY was immunoprecipitated overnight at 4 °C after the addition of 2 μl of anti-CPY antisera (29) and protein A-Sepharose beads (100 μl of a 20% (w/v) suspension). Immunocomplexes were washed twice in a buffer containing 10 mM Tris-HCl, pH 8.0, 0.2 mM NaCl, 0.05% Triton X-100, and 0.1% SDS at room temperature, boiled in Laemmli sample buffer containing 25 mM DTT, and analyzed by SDS-PAGE. Endo H treatment was done by boiling the immunoprecipitated samples in a buffer containing 0.5% SDS and 100 mM sodium acetate, pH 5.5, thereafter adding an equal volume of 100 mM sodium acetate, pH 5.5, and 5 × 10⁻⁴ units of Endo H, and incubation overnight at 37 °C.

RESULTS

Purification of Rat Liver Glucosidase II—In order to obtain enough purified glucosidase II that would enable us to get sequence information and to perform functional studies, we
developed a purification protocol that emphasized rapid processing and high purity (Table I; see “Experimental Procedures” for details). We used rat livers as the starting material. Microsomes were isolated by standard subcellular fractionation in a solution containing 0.25 M sucrose, 5 mM 2-mercaptoethanol, and 5 mM EDTA. A soluble extract was prepared from the microsomal fraction using Triton X-100 and fractionated by DEAE-cellulose, concanavalin A-Sepharose, and Mono Q. When fractions containing glucosidase II activity from the Mono Q column were analyzed by SDS-PAGE, they contained two bands with apparent molecular masses of 110 and 80 kDa (Fig. 1A). Since a variety of experiments described below strongly indicated that the enzyme was a heterooligomer containing these polypeptides, we will call the larger subunit α and the smaller β.

Attempts to separate the α and β chains under conditions that preserved enzymatic activity were unsuccessful. As shown in Fig. 2, the same two proteins were found to co-elute with the glucosidase II activity in all of the high resolution chromatography columns tested including ion exchange, gel filtration, hydrophobic interaction, and hydroxyapatite chromatography (Fig. 2). Therefore, in the purification table only the Mono Q step is included, since the material obtained after it could no longer be fractionated.

Different solubilization procedures were tested to rule out the possibility that the proteins would have associated as a result of a particular solubilization method. For this, rat liver microsomes were extracted with different concentrations of Triton X-100 (0.05, 0.1, 0.2, and 0.5%) or Lubrol (0.1 and 0.5%). Also, microsomes were extracted with sodium cholate (0.1 and 0.5%) followed by ammonium sulfate precipitation. The soluble material obtained from each individual solubilization trial was subjected to all the fractionation steps including DEAE-cellulose, concanavalin A-Sepharose, Mono Q, and Superdex 200. In all cases, the same two protein bands were present in equal proportions no matter which solubilization procedure was used.

The co-purification of the two proteins was therefore not a consequence of a particular method of releasing the luminal content from the microsomes.

Specificity of the Purified Glucosidase II—The specificity of the purified enzyme was tested on a panel of substrates (Table II). Both Glc3Man9GlcNAc and Glc2Man9GlcNAc, but not Glc1Man9GlcNAc, were hydrolyzed by the purified enzyme. It had a neutral pH optimum, but the activity was not affected by Ca2+, Mn2+, or Mg2+ (up to 20 mM), and it was not inhibited by EDTA (not shown). It was, however, effectively inhibited by the glucose analogs castanospermine and 1-deoxynojirimycin (I50 ~ 15 μM) known to block glucosidase II (30). The enzymatic properties were thus identical to those previously described (1, 2, 30), suggesting that the presence of the β chain did not alter the specificity of the purified enzyme.

Characterization of the Purified Enzyme—We were unable to separate the two polypeptides from each other or from the enzymatic activity by a variety of separation techniques (Fig. 2). Also, during non-denaturing polyacrylamide gel electrophoresis the two polypeptides migrated together (Fig. 1B). Upon sucrose gradient velocity centrifugation, a single peak of activity was found, containing α and β chains in the same proportion throughout the peak. The sedimentation coefficient (s20,w) determined by the method of Martin and Ames was 6.5 S (31). Gel filtration experiments indicated an approximate diffusion coefficient of 4.0 × 107 cm2/s. Combined with a calculated partial specific volume of 0.72, a molecular mass of 150,000 Da could be calculated for the active enzyme, indicating that it was a discrete multimer and not an aggregate of α and β subunits.

The possibility that the β chain would be a proteolytic fragment of the α chain was unlikely on several accounts. Western blots showed that only the α band stained with a polyclonal antiserum made against the 110-kDa polypeptide of pig liver glucosidase II (3). Peptide maps of α and β chains were completely different (not shown). Finally, a comparison of SDS-PAGE mobilities before and after reduction indicated that the β subunit contained intrachain disulfides. It migrated faster in nonreduced form than in the reduced form, while α had a similar mobility under reducing and nonreducing conditions (Fig. 1A). This electrophoretic analysis also revealed that the α and β subunits were not connected by disulfide bonds.

In conclusion, the results indicated that glucosidase II was a dimeric protein of about 150 kDa containing two different polypeptides. That α and β subunits were distinct gene products was further confirmed by their primary sequences shown in Figs. 3 and 4.

**Primary Sequence of a Subunit**—Peptide sequences from the N terminus and from 10 internal positions were determined for
the α subunit. As shown in Fig. 3, a BLAST search (32) identified a human cDNA of unknown function recently deposited in data bases (accession number D42041) to which all of the 11 peptide sequences obtained from the α chain, including the N and C termini, could be matched. The cDNA sequence available lacks an initiator ATG but predicts a hydrophobic signal sequence followed by a soluble protein of 912 amino acids (103,596 Da) in close agreement with the properties of the α subunit. It has two potential N-glycosylation sites, of which at least one appeared to be glycosylated based on concanavalin A-Sepharose binding (Table I) and sensitivity to Endo H (not shown). The sequence failed to show any putative hydrophobic transmembrane elements or a C-terminal KDEL ER retention/retrieval sequence.

Analysis of the primary sequence revealed the presence of zones diagnostic of family 31 and family 9 glucosidases (Fig. 3) (33). It also showed similarity to other known glucosidases: Hordeum vulgare α-glucosidase (U22450), human lysosomal α-glucosidase (Y00839), Schwannomyces occidentalis glucoamylase (M60207), human, rabbit and rat sucrase-isomaltase (X63597, M14046, and L25928), Aspergillus oryzae α-glucosidase (D45179), and Candida tsukubaensis α-glucosidase.

Table II

Specificity of purified glucosidase II and of microsomes from yeast strains employed

In all cases the reactions were conducted in a 100-μl total volume, and the [14C]glucose release was measured as in Ref. 21. In the case of pure glucosidase II from rat liver, 0.1 μg of pure enzyme was incubated for 10 min at 37 °C. Yeast microsomal membranes were prepared as in Ref. 49. Reactions contained 300–600 μg of total protein, 0.6% Triton X-100, and 800–2500 cpm of the corresponding substrates and were incubated for 60 min at 30 °C. The values obtained from blank incubations lacking enzyme, (−50 cpm) were subtracted.

| Enzyme source | [14C]Glucose-Glc1Man4GlcNAc | [14C]Glucose-Glc2Man4GlcNAc | [14C]Glucose-Glc3Man4GlcNAc |
|---------------|-----------------------------|-----------------------------|-----------------------------|
| Pure rat liver glucosidase II | 2045 | 545 | 0 |
| NY914 (wild type) microsomes | 2037 | 504 | 466 |
| gls1–1 microsomes | 1985 | 468 | 0 |
| NY914Δgls2Δ2 microsomes | 0 | 0 | 387 |
| NY914Δgls2 + NY914 microsomes | 2185 | ND | ND |

ND, not determined.

'a' gls2 stands for gene Z36098, the S. cerevisiae homologue of the catalytic subunit of mammalian glucosidase II.

Fig. 2. Purification of rat liver glucosidase II. A–D, chromatographic analysis of purified rat liver glucosidase II. Open circles with dotted lines depict enzymatic activity. Solid lines represent absorbance at 280 nm. Insets show SDS-PAGE analysis of fractions across the activity peaks (5 μl of each fraction were loaded per lane for the Mono Q and Superdex 200; 10 μl were loaded for the phenyl and hydroxylapatite columns). Gels were stained with Coomassie Blue. In A, arrows represent the elution volume for void volume (V0), thyroglobulin (660 kDa) (1), ferritin (450 kDa) (2), β-amylase (200 kDa) (3), catalase (240 kDa) (4), aldolase (150 kDa) (5), and bovine serum albumin (68 kDa) (6).
It therefore seemed likely that the α subunit contained the catalytic domain of the enzyme. A gene from chromosome II of *S. cerevisiae* (Z36098; Ref. 34) was very similar to the human cDNA across the entire open reading frame (59% similarity, 39% identity, Fig. 3). This sequence showed an initiator ATG and a putative signal sequence followed by a hydrophilic protein without apparent transmembrane domains or ER retention/retrieval signals. That this gene, which we will refer to as *gls2*, indeed encoded the yeast functional homologue of the catalytic subunit of glycoprotein processing glucosidase II was shown by gene disruption experiments described below.

Identification of the *S. cerevisiae* Functional Homologue of Mammalian Glucosidase II Catalytic Subunit (α)—To verify that the sequence identified in *S. cerevisiae* encoded a functional homologue of the mammalian α subunit, the entire open reading frame of gene Z36098 was disrupted by homologous recombination, and the carbohydrate processing of glycoproteins was examined in the disrupted strains. Both a diploid strain with one genomic copy disrupted and a haploid strain completely lacking the gene were obtained. The incorporation of the *URA3* marker in the correct position of the Z36098 locus was verified by polymerase chain reaction. Both strains were viable, and no growth defect could be detected in a temperature range between 14 and 37 °C. This was consistent with earlier observations that a yeast mutant lacking glucosidase I activity grew normally (35).
To determine whether trimming of the two α1–3-linked glucoses was affected in the disrupted haploid strain, two approaches were employed. First, the presence of glucosidase II activity was assessed by a specific enzymatic assay. Second, the mobility of two endogenous yeast glycoproteins, CPY and PDI, were analyzed by SDS-PAGE and compared with their mobility in wild type and a glucosidase I-deficient strain \( \text{gls1}^{\text{–1}} \) (35).

As shown in Table II, using microsomal membranes from the glucosidase II \( \alpha \) chain deletion strain, no hydrolysis of the specific substrates of glucosidase II, Glc1Man9GlcNAc, or Glc2Man9GlcNAc was observed, whereas removal of the outermost Glc from Glc3Man9GlcNAc was unaffected. Using membranes from both the parental wild type strain and from the \( \text{gls1}^{\text{–1}} \) strain, degradation of Glc2Man9GlcNAc and Glc1Man9-GlcNAc was detected. A mixing experiment employing microsomes from the disrupted and the parental strains showed that no unspecific inhibition occurred. These results indicated that the deletion of the Z36098 gene specifically abolished glucosidase II but not glucosidase I activity.

Western blot analysis showed that PDI from the \( \text{gls1}^{\text{–1}} \) strain migrated slower than PDI from the wild type strain (Fig. 5A, lanes 1 and 3). PDI from the haploid strain lacking glucosidase II \( \alpha \) subunit migrated slightly faster than the completely untrimmed form but clearly slower than that of the wild type strain (lane 2). Treatment of the samples with Endo H confirmed that the mobility differences were due to varying degrees of carbohydrate trimming. A similar analysis of the trimming of CPY showed that partial glucose removal from N-linked oligosaccharides was not restricted to PDI. In this case, the cells were radiolabeled in the presence of dithiothreitol to prevent the exit of CPY from the ER (36), and CPY was immuno precipitated from the cell lysate. As shown in Fig. 5B (lanes 2 and 3) a slight increase in mobility was observed for CPY from the disrupted strain compared with the \( \text{gls1}^{\text{–1}} \) mutant, and this difference could be abolished by Endo H treatment (Fig. 5B, lanes 4–6).

### Primary Sequence of \( \beta \) Subunit—Peptide sequences from the N terminus and from six internal positions were determined for the \( \beta \) subunit of human glucosidase II (J03075). The sequences below show the peptide sequences determined from the rat protein. × denotes positions not unambiguously determined in the corresponding cycle during Edman degradation. The arrow indicates the N-terminal sequence of the purified protein. The putative EF-hand calcium binding loops are boxed.

| Human | 1 MLLPLLLPPP WCMAVEKRP RGVSLLNHSF YDESSKPTCL DGZATIPFDQ VNDDYDCDKE | Rat | VKEKRP RGVSLLNHSF Y |
|-------|---------------------------------------------------------------|-----|-----------------|
| Human | 61 GSDEPPTAACC PNGSFHCCTT GYRPFLYPGDN RVDQVCDCOC DOTDEYNSGV ICERTCKEG |     |                 |
| Human | 121 RKEKISIQQOM ADVTRKFGRL KKLINDMKK AREKQKQKLI EIQACQELKE DQVEMRLTVK | Rat | ESIQQL AEBTR ILIREW |
hydrophilic protein of 514 amino acids (57,682 Da) with no obvious membrane-spanning regions. Two putative EF-hand motifs for high affinity Ca\(^{2+}\) binding loops were identified (Fig. 4) (39). A conspicuous stretch of consecutive glutamic acids probably explained why this 57-kDa protein migrated with an apparent molecular mass of 80 kDa on SDS-PAGE. Anomalously slow migration has been previously observed for other proteins (including calnexin (40) and calreticulin (41)) that contain long stretches of negatively charged residues. A clustering of cysteine residues at the N and C termini is consistent with the presence of large cysteine-induced loops, explaining the differential migration of the β subunit under reducing and nonreducing conditions (Fig. 1A). The presence of the C-terminal HDEL sequence suggests that this subunit mediates retention/retrieval of the enzyme to the ER.

A bovine homologue of the human β subunit was recently deposited in data bases (accession number U49178) that is virtually identical to the human cDNA (93% similarity, 87% identity). We were unable to identify a yeast homologue of the mammalian β subunit from the complete S. cerevisiae genome.

**DISCUSSION**

Our results indicated that glucosidase II is an oligomer of two different subunits α and β. The α subunit shares homology with many glucosidases and represents the catalytic domain, while the β subunit carries ER retention/retrieval signals and is likely to mediate the ER localization of the enzyme.

Previous reports have suggested that glucosidase II is a polypeptide of 100–120 kDa (15, 16, 18). In terms of molecular weight and immunoreactivity, this corresponds to our α subunit. None of the previous reports acknowledged the presence of a second subunit in the intact enzyme. A polypeptide with an apparent molecular mass of approximately 80 kDa was, however, also visible in previous reports but was either disregarded (15) or considered a contaminant (16). We began to suspect that the β subunit was not a contaminant but a true constituent of the native enzyme when we were unable to separate it from the α subunit or from the enzymatic activity under a variety of conditions short of denaturation. The two proteins co-eluted in all the chromatography columns tested, and they ran together as a discrete complex during gel filtration. They also migrated together in non-denaturing electrophoresis and sedimented as a defined complex during ultracentrifugation. In short, glucosidase II behaved like a discrete heterooligomeric enzyme complex.

The relative proportion of the α and β subunits remained constant throughout the entire purification process. Amino acid analysis of the individual subunits gave an α/β ratio of 1.3, suggesting an actual stoichiometry of 1:1. The behavior of the purified enzyme in gel filtration chromatography and sedimentation suggests that the oligomeric structure is probably αβ. The enzyme is entirely soluble in the absence of detergents, with little tendency for aggregation.

Sequence analysis of peptides derived from α and β chains allowed us to identify the human cDNAs coding for both polypeptides. The biochemical properties were consistent with the primary sequences deduced from the corresponding cDNAs. The β subunit showed no homology with other known proteins. The sequence of the α subunit contained diagnostic sequences of the so-called family 31 and 9 glucosidases (33), indicating that it represents the catalytic subunit. It shares similarity with other glucosidases mainly in the C-terminal half, with no obvious homologies to other proteins observed in the N-terminal part. Interestingly, neither subunit had any homology with ER glucosidase I (42), suggesting that the two ER glucosidases have evolved independently.

While known from electron microscopic analysis to be located in the ER (3), the α chain lacked ER retention/retrieval signals. This implied that it is either retained in the ER by a yet unidentified mechanism or that it is associated with another entity responsible for ER localization. The β subunit had not only an HDEL sequence at its C terminus but also a long negatively charged sequence similar to those found in calreticulin and other lumenal ER proteins. These regions are typically sites of low affinity calcium binding, and they are believed to serve a role in luminal protein retention (43). Prolyl 4-hydroxylase (44) and the triglyceride transfer protein (45) are examples of other resident ER proteins that lack retention signals. Both of these use PDI, which possesses the correct signals for retention in the ER, as a stably associated additional subunit.

In a yeast α chain knock-out strain, we found no glucosidase II activity. Moreover, we could show that two glycoproteins, PDI and CPY, were incompletely trimmed in the ER, while their synthesis and stability remained unaffected. This provided direct evidence that the sequence assignment for the α chain was correct and that the gene product was necessary for glucosidase II activity. The existence of cell lines defective in glucosidase II (Phar 2.7 (46)) and glucosidase I (Chinese hamster ovary Loc 23 (47) and S. cerevisiae strain gls1–1 (35)) suggests that glucose trimming is not essential for viability. Results presented here demonstrate unambiguously that lack of the glucosidase II gene product is compatible with cell viability in S. cerevisiae. We were unable, however, to identify a homologue of the mammalian β subunit from the complete yeast genome. Therefore, the existence of a functional subunit with the characteristics of the mammalian β chain in yeast remains to be established.

Why is the ER glucosidase II a heterooligomer and larger than other α-glucosidases? A likely explanation is that, in addition to its oligosaccharide substrate, it needs to interact with other molecules. These may include calnexin and calreticulin, which bind some of the substrate molecules. While it is presently not known whether the monoglucosylated glycans in the chaperone complexes can serve as substrates for glucosidase II, it is clear that the glucose residues have to be hydrolyzed to dissociate the glycoproteins from calnexin (7). This means that glucosidase II may need to interact with calnexin and calreticulin. In addition, as already mentioned, other interactions are needed for retention in the ER. Finally, we have recently shown that calnexin and calreticulin associate with growing nascent chains of influenza HA in living cells (48). This means that glucosidase I and II both have to have access to glycopeptides just as they are entering the ER lumen. It is thus possible that both glucose-trimming enzymes are part of a large complex of proteins surrounding the translocation sites.

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