The Lec23 Chinese Hamster Ovary Mutant Is a Sensitive Host for Detecting Mutations in α-Glucosidase I That Give Rise to Congenital Disorder of Glycosylation IIb (CDG IIb)*

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Lec23 Chinese hamster ovary cells are defective in α-glucosidase I activity, which removes the distal α(1,2)-linked glucose residue from GlcMan7GlcNAc2 moieties attached to glycoproteins in the endoplasmic reticulum. Mutations in the human GCS1 gene give rise to the congenital disorder of glycosylation termed CDG IIb. Lec23 mutant cells have been shown to alter lectin binding and to synthesize predominantly oligomannosyl N-glycans on endogenous glycoproteins. A single point mutation (TCC to TTC; Ser to Phe) was identified in Lec23 Gcs1 cDNA and genomic DNA. Serine at the analogous position is highly conserved in all GCS1 gene homologues. A human GCS1 cDNA reverted the Lec23 phenotype, whereas GCS1 cDNA carrying the lec23 mutation (S440F in human) did not. By contrast, GCS1 cDNA with an R486T or F652L CDG IIb mutation gave substantial rescue of the Lec23 phenotype. Nevertheless, in vitro assays of each enzyme gave no detectable α-glucosidase I activity. Clearly the R486T and F652L GCS1 mutations are only mildly debilitating in an intact cell, whereas the S440F mutation largely inactivates α-glucosidase I both in vitro and in vivo. However, the S440F α-glucosidase I may have a small amount of α-glucosidase I activity in vivo based on the low levels of complex N-glycans in Lec23. A sensitive test for complex N-glycans showed the presence of polysialic acid on the neural cell adhesion molecule. The Lec23 Chinese hamster ovary mutant represents a sensitive host for detecting a wide range of mutations in human GCS1 that give rise to CDG IIb.

There are a growing number of human diseases that arise from altered glycosylation of glycoproteins or lipid-linked oligosaccharides and are termed congenital disorders of glycosylation (CDG)1 (1, 2). In addition, there are a number of myopathies due to defective O-glycan synthesis on α-dystroglycan (3, 4) and diseases that disrupt the synthesis of proteoglycans (5). As disease mutations are identified, it becomes important to characterize the altered biochemical properties of mutant genes and to investigate ways in which enzyme activity may be modulated. Such studies are best performed in a cell that is null for the activity under investigation. Cells that lack a glycosylation enzyme activity may be derived from a particular patient or a mutant mouse or mutant cells selected in culture. The latter are most desirable because fibroblasts isolated from humans or mice often have a finite lifetime, are not easy to grow, or are difficult to transfet. By contrast, cultured Chinese hamster ovary (CHO) cells are well characterized, are easy to culture and transfet, and have many characterized glycosylation mutants (6–8). In this paper we describe the molecular basis of the Lec23 CHO mutant and show that it is an excellent host for characterizing mutations in α-glucosidase I that give rise to the human congenital disorder of glycosylation CDG IIb.

Lec23 cells were obtained following a single step selection for lectin resistance from a mutagenized population of CHO cells (9). The cells are highly resistant to the leukoagglutinin from Phaseolus vulgaris (L-PHA) and to wheat germ agglutinin (WGA) and are hypersensitive to concanavalin A (Con A). Complementation analysis showed that the lec23 mutation is recessive, and biochemical analysis revealed defective α-glucosidase I activity (10). α-Glucosidase I initiates the processing of N-glycans by removing the distal α(1,2)Glc residue from GlcMan7GlcNAc2Asn in the endoplasmic reticulum (11, 12). The G glycoprotein of vesicular stomatitis virus obtained from Lec23 mutant cells has predominantly oligomannosyl N-glycans with three terminal glucose (10). Lec23 cells have been used to study endoplasmic reticulum chaperones, such as calnexin, calreticulin, and ERP57, that recognize monoglycosylated oligomannosyl N-glycans and aid in the folding of glycoproteins (13–18). Lec23 cells have also been useful in characterizing Golgi endomannosidase (19, 20) and the biological roles of N-glycans in human immunodeficiency virus infection (21, 22). Thus it was important to determine the molecular basis of the mutation in Lec23 cells that might be in the Gcs1 gene that encodes α-glucosidase I or in a regulatory gene. In either case, Lec23 cells would be useful in understanding mutations that give rise to an α-glucosidase I deficiency and CDG IIb (23, 24). Here we report that Lec23 CHO cells express a leukoagglutinin from Phaseolus vulgaris; PSA, polysialic acid; FITC, fluorescein isothiocyanate; BSA, bovine serum albumin; PBS, phosphate-buffered saline; MALDI-TOF, matrix-assisted laser desorption/ ionization time-of-flight; PIPES, 1,4-piperazinediethanesulfonic acid; mAb, monoclonal antibody; Endo H, endoglycosidase H; FACS, fluorescence-activated cytometry; PNGase F, peptide:N-glycosidase F.

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‡ This abbreviation is used as: CDG, congenital disorders of glycosylation; N-CAM, neural cell adhesion molecule; CHO, Chinese hamster ovary; Con A, concanavalin A; WGA, wheat germ agglutinin; L-PHA, matrix-assisted laser desorption/ ionization time-of-flight.
point mutation in the Gcs1 gene that generates a missense mutation and largely inactivates α-glucosidase I. Lec23 cells are shown to be sensitive hosts for characterizing mutations in human GCS1 that give rise to CDG IIb.

**EXPERIMENTAL PROCEDURES**

**Materials**—Mouse monoclonal anti-e2,8-polyasialic acid (PSA) IgG2a antibody (35) was a gift from Dr. Rita Gerardy-Schahn (Zentrum Biochemie). N-CAM13 (BD Biosciences) is a monoclonal IgG1 antibody recognizing the extracellular domain of mouse N-CAM. FITC-conjugated anti-mouse IgG+M antibody (Zymed Laboratories Inc.) was used as a secondary antibody. FLAG-tagged proteins were detected by anti-FLAG M2 antibody from Sigma. Lectins including Con A, WGA, L-PHA, and ricin with and without biotinylation were purchased from Vector Laboratories (Burlingame, CA). Restriction enzymes and buffers were from Roche Diagnostics GmbH (Mannheim, Germany), Invitrogen, and New England Biolabs (Beverly, MA). Hybond N+ membrane was from Amersham Biosciences. DNA probe was labeled with Prime-it II random primer labeling kits (Stratagene) with [α-32P]dCTP (PerkinElmer Life Sciences). Synthetic oligonucleotides, Superscript II, and dNTPs were purchased from Invitrogen. [3H]-Labeled Glic-ManGlicNACAsn in Glic residues was prepared as described (10). Other chemicals and reagents were from Sigma and Fisher.

**Cell Lines, Cell Culture, and Transfection**—Parent CHO (Pro 5), Lec23 (Pro Lec23.11C) defective in α-glucosidase I (9, 10), and Lec2 (Pro Lec2.4C) defective in the CMP-sialic acid transporter (26, 27) were routinely cultured in complete medium (α minimum Eagle’s medium (Invitrogen)) containing 10% fetal calf serum (Gemini) in suspension or monolayer culture at 37 °C.

To transfet expression constructs, cells were cultured on a 24-well plate for 1 day to ~90% confluence, washed once with Opti-MEM I medium (Invitrogen), and incubated in 0.5 ml Opti-MEM I medium containing 3% fetal calf serum. Plasmid DNA (2 μg) and LipofectAMINE 2000 from Invitrogen (25 μg/ml) were mixed with 50 μl of Opti-MEM I in separate tubes. After 5 min at room temperature, plasmid DNA and LipofectAMINE 2000 were mixed, incubated for 20 min, and added to cells. After 12–24 h at 37 °C, cells were detached with trypsin/EDTA and cultured in complete medium (Invitrogen) for 1 day. To detect N-CAM or PSA, cells were incubated with 4 volumes of 1% BSA/PBS, cells were incubated with 200-fold anti-PSA mAb 735 in 1% BSA/PBS on ice for 15 min. After washing once with 1% BSA/PBS, cells were incubated with 200-fold diluted anti-mouse IgG-FITC (Zymed Laboratories Inc.) in 1% BSA/PBS on ice for 15 min. After washing once with 4 volumes of 1% BSA/PBS, cells were incubated with 200-fold diluted anti-mouse IgG-FITC (Zymed Laboratories Inc.) in 1% BSA/PBS on ice for 15 min. After washing once with 4 volumes of 1% BSA/PBS, cells were incubated on a FACScan instrument (BD Biosciences). Stained cells were gated based on size.

**Matrix-assisted Laser Desorption Ionization Time-of-flight Mass Spectrometry**—Glycoproteins were extracted from parent and Lec23 CHO cells in 1.5% Triton X-100 containing protease inhibitors (Roche Applied Science), and 500 μg of protein was treated with peptideN-glycosidase F (PNGase F) or endoglycosidase H (Endo H) (New England Biolabs) as described (28). Released N-glycans were methylated under conditions that specifically methylated sialic acid residues only so they could be detected in the positive ion mode (29). Subsequent sample purification was by ion exchange chromatography as described (29), followed by polygraphite carbon column chromatography as described (28). MALDI-TOF mass spectrometry was performed on a Voyager DE Biospectrometry work system (Perceptive Biosystem) equipped with delayed nitrogen laser and a xenon flash lamp. Mass glycopeptides were used for external calibration for mass assignment of ions, and α-arabinobiosamine (30) was used as the matrix in the positive ion mode for the analysis of neutral oligosaccharides.

α-Glucosidase I and β-Galactosyltransferase 1 Assays—Parent and Lec23 CHO cell extracts prepared in 1.5% Triton X-100 with protease inhibitors (Roche Applied Science) were assayed for α-glucosidase I activity using [14C]Glc[Mana]GlcNACAsn as substrate as described previously (31). Northern Blot Analysis—Total RNA (30 μg) was extracted using TRIzol (Invitrogen) and separated in a 1% agarose-denaturing gel. After transfer to a Hybond N+ membrane, a 1.5-kb probe was PCR-amplified from pCR-CHO-GCS1(3') plasmid using CHO-GCS2F (5'-attgcggc-gaatacctacgaag-3') and CHO-GCS4R (5'-agggactcagatggctgc-3') primers and purified from a 1% agarose gel. The PCR products were radiolabeled using the Random-It priming kit (Stratagene). The membrane was incubated in prehybridization solution (0.5× MPPES, 0.1 × NaCl, 0.05 × NaPO4, pH 7.0, 10 mM EDTA, 5% SDS, 0.1 mg/ml denatured salmon sperm DNA) at 60 °C for 2 h before adding the probe, and the incubation was continued at 60 °C overnight. Washing was performed twice in 2× SSC, 0.1% SDS and twice in 0.2× SSC, 0.1% SDS before exposure to x-ray film for 2 days at ~80 °C. For stripping, the membrane was soaked in 0.1% SDS in diethyl pyrocarbonate-treated water, put into a roto mixer, and stored at ~20 °C before probing with a glyceraldehyde-3-phosphate dehydrogenase probe to determine relative loading and RNA integrity.

Cloning of CHO Gcs1 cDNA—To clone Chinese hamster Gcs1, subpools of 5,000 cDNA clones were made from a CHO-K1 cell library in pSPORT (a gift from Drs. Osamu Kuge and Masahiro Nishijima, Institute of Infectious Diseases, Tokyo, Japan) and screened by PCR with the forward primer hGCS3F(SalI) and the reverse vector primer, sequenced, and found to contain poly(A) signals. This fragment of 2.1 kb was cloned into the pCR2.1 vector (Invitrogen) and named pCR-CHO-Gcs1(3').

Construction of a Human Gcs1 cDNA—IMAGE clone 3637073, which contains an alternatively spliced human GCS1 cDNA at the 5′ end, was purchased from the IMAGE Consortium. To construct a functional GCS1 cDNA, the 3′ region was amplified using hGCS1F(5'-agtgcggcctggtctggtc-3') and hGCS1R(XbaI) (5′-gctgatggctggattggTACggagcagatacgggg-3') primers and subcloned into pCR2.1 (Invitrogen). A 0.35-kb EcoRI-KpnI fragment from IMAGE clone 3637073 and a 2.3-kb KpnI-XbaI fragment cloned in pCR2.1 were ligated into pcDNA3.1(+) that had been digested with EcoRI and XbaI and named pME-3FLAG-hGCS1. To clone Chinese hamster Gcs1, the Gcsg1 coding region was amplified by PCR using hGCS3F(SalI) (5′-agtgcggcctggtctggtc-3') and hGCS1R(XbaI) primers. This fragment was cut at SalI and XbaI sites, cloned into pME-3FLAG(32) and named pME-3FLAG-hGCS1. pcDNA3.1(+) and pME-3FLAG-hGCS1 were sequenced at the ATCC DNA sequencing facility.

Site-directed Mutagenesis of Human FLAG-tagged GCS1—Mutants of FLAG-tagged human GCS1 were generated using an oligonucleotide-directed mutagenesis method (Stratagene) with pME-3FLAG-hGCS1 to generate the lec23 mutation S440F and the human CDG IIb mutations R486T and D5576E. Point mutants were constructed with hGCS1F (5′-tttaagagctgcctgcAgtgctgtcagatggc-3') and hGCS1R (5′-gctgatggctggattggTACggagcagatacgggg-3') using the overlap extension method. The PCR products were sequenced for purity. To introduce the GCS1 mutation, the 3′ end of the human GCS1 coding sequence was amplified by PCR using hGCS3F(SalI) (5′-agtgcggcctggtctggtc-3') and hGCS1R(XbaI) (5′-agggactcagatggctgc-3') primers and subcloned into pcDNA3(−) using the EcoRI and SalI restriction sites. For introduction of the GCS1 mutation, the 5′ end of the human GCS1 coding sequence was amplified by PCR using hGCS3F(SalI) (5′-agtgcggcctggtctggtc-3') and hGCS1R(XbaI) (5′-agggactcagatggctgc-3') primer and subcloned into pcDNA3(−) using the EcoRI and SalI restriction sites.

Western Blot Analysis—Lec23 cells (1.5× 106) were incubated in a 6-well plate in complete medium for 12 h. Cells were washed once with Opti-MEM I, and Opti-MEM I with 3% fetal calf serum was added. pME-3FLAG-hGCS1 or GCS1 mutant cDNA (10 μg/well) and 10 μl of LipofectAMINE 2000 were mixed with 250 μl of Opti-MEM I, respectively. After 5 min at room temperature, diluted plasmids and LipofectAMINE 2000 were mixed together. After 20 min, DNA liposomes were added to the cells in a 5% CO2 incubator. Cells were detached using trypsin/EDTA and cultured in complete medium for 1 day. To detect FLAG-tagged GCS1, cells (5× 106) were dissolved in 1 ml of 1% Nonidet P-40 in TNE buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA) for 1 h. After centrifugation at 15,000 × g for 10 min, tagged proteins were immunoprecipitated from the supernatant with biotinylated anti-FLAG M2 antibody-conjugated beads (Sigma) for 1 h. After washing three times with 1% Nonidet P-40 in TNE buffer,
proteins were released from beads by boiling in sample buffer, separated in 7.5% acrylamide gel by SDS-PAGE, and transferred to polyvinylidene difluoride membrane. Western blot analysis was performed with biotinylated anti-FLAG M2 antibody (1/2000) and horseradish peroxidase-conjugated avidin (Pierce) (1/5000) as described (33).

To detect complex N-glycans on N-CAM, cells (1 × 10^7) were extracted in 1 ml of 1% Nonidet P-40 in TNE. Supernatants obtained after 1 h of incubation on ice and centrifugation at 10,000 g for 5 min were immunoprecipitated with anti-PSA mAb 735 and protein G beads (Amersham Biosciences). After adding sample buffer and boiling, proteins were separated on a 6% acrylamide gel by SDS-PAGE and transferred to polyvinylidene difluoride membrane. N-glycans were detected with biotinylated L-PHA (10 μg/ml) (Vector) and horseradish peroxidase-conjugated avidin (1/2000). PSA modified N-CAM was detected on the same membrane after incubation in stripping buffer (62.5 mM Tris (pH 6.8), 2% SDS, 100 mM 2-mercaptoethanol) at 55 °C for 30 min followed by washing in 5 mM Tris (pH 8.0) containing 1% Nonidet P-40. PSA on proteins was detected by an enhanced chemiluminescence kit and exposure to x-ray film.

RESULTS

Lec23 Mutants Are Readily Differentiated from CHO Cells by Lectin Binding—Lec23 cells were previously distinguished from CHO cells by a lectin resistance cytotoxicity test as shown in Fig. 1 (9). They are highly resistant to L-PHA and WGA, lectins that bind to complex N-glycans, and are hypersensitive to Con A, a lectin that binds best to oligomannosyl N-glycans (34). To determine whether resistance to lectins reflects a reduction in lectin binding and hypersensitivity reflects increased lectin binding, Lec23 and CHO cells were examined by

![Lectin toxicity and binding](image-url)
Lec23 CHO Has a Missense Mutation in α-Glucosidase I

N-glycans reflect the existence of an alternative processing pathway. The major N-glycan released by Endo H was GlcM8Gn1 (Fig. 2B and Table I), and there were minor species lacking Glc, which indicates a small amount of α-glucosidase I activity in Lec23 or an alternative pathway. Although Man9GlcNAc1 lacking Glc might theoretically arise from the action of a Golgi endomannosidase (37), there is no evidence that CHO or Lec23 cells have endomannosidase activity (20, 38).

**Point Mutation of Lec23 Mutant Cells in α-Glucosidase I—**Previous studies showed that Lec23 mutant cells have no detectable α-glucosidase I activity in vitro (10, 20). To investigate the expression of the Gcs1 gene that encodes α-glucosidase I, Northern blot analysis of total RNA was performed. Lec23 and CHO cells had Gcs1 transcripts of similar size (Fig. 3A). Therefore, a mutant Gcs1 gene in Lec23 cells may have a small deletion, insertion, or point mutation, or the mutation might lie in a regulatory region or regulatory gene.

To isolate Gcs1 cDNAs for sequencing, a CHO cDNA library was screened by PCR using degenerate primers designed from mouse and rat Gcs1 3′-coding regions. A partial hamster sequence was obtained that corresponded to exons 2, 3, and 4 of mammalian Gcs1 genes (Fig. 3B). The hamster Gcs1 cDNA sequence was 84, 90, and 90% identical to human, mouse, and rat cDNA, respectively. The deduced amino acid sequence was 91% identical to mouse Gcs1. Unfortunately, attempts to obtain the 5′ CHO Gcs1 coding sequence were not successful for two reasons. First, we could not amplify exon 1 from the pSPORT PCR library using vector and 5′-coding region primers. It is likely that the 5′ end of the Gcs1 cDNA is not represented in the library because it was generated following digestion with NotI for cloning purposes, and human, mouse, and rat Gcs1 sequences contain one or two NotI sites in exon 1. Second, attempts at 5′ rapid amplification of cDNA ends were probably not successful because of the high GC content of Gcs1 5′-coding regions.

Reverse transcription-PCR was used with primers spanning the partial CHO cDNA to obtain Gcs1 cDNAs from CHO and Lec23 total RNA. Direct sequencing of these PCR products identified a single nucleotide change of C to T at nucleotide 964 in the partial hamster Gcs1. We found the same mutation in a reverse transcription-PCR product from Lec23 cells. The position corresponds to Ser-440 in the human Gcs1 sequence as noted in Fig. 3A. Sequencing of reverse transcription-PCR products from Lec23 cells gave no evidence for the presence of Gcs1 transcripts with the parental CHO
sequence. In addition, only the mutant sequence was present in genomic DNA as determined by sequencing of genomic DNA PCR products. These data are consistent with previous biochemical studies showing that Lec23 CHO cells do not have \( \alpha \)-glucosidase I activity that could arise from a wild-type allele (10, 20).

Reduction of \( \alpha \)-Glucosidase I Activity by the Lec23 Gcs1 Mutation—A sensitive test of \( \alpha \)-glucosidase I activity is to convert the reduced L-PHA binding of Lec23 cells to parental levels (Fig. 1). This was accomplished by transient transfection of a human GCS1 cDNA into Lec23 cells (Fig. 4). About 30% of the transfected cells bound L-PHA over a wide range, which was consistent with varying levels of expression in the transfected population. A small fraction of cells bound more L-PHA than CHO control cells, indicating the effects of overexpression of \( \alpha \)-glucosidase I. No increase in L-PHA binding was observed with vector control. By contrast, none of the Lec23 cells expressing a mutant GCS1 cDNA carrying the lec23 mutation (S440F) had increased binding of L-PHA, although the amount of enzyme expressed was shown by Western blot analysis to be equivalent in both cases (Fig. 4A). Therefore an overexpressed human \( \alpha \)-glucosidase I cDNA with the S440F mutation did not rescue the Lec23 phenotype.

The Lec23 Mutant Reveals Activity of GCS1 Mutant Alleles Identified in CDG IIb Patients—The Lec23 Gcs1 mutation is located in exon 4, the exon thought to encode the catalytic activity of \( \alpha \)-glucosidase I (40, 41). Two mutations in the human GCS1 gene (R486T and F652L) are located in exon 4 of both patients (23). Biochemical analyses of these mutations in fibroblasts or introduced into a human GCS1 cDNA and transfected into COS...
cells indicated that both mutations reduce enzyme activity ≥97% (24). Therefore a human cDNA carrying either of these α-glucosidase I mutations would be expected to behave like the Lec23 mutant cDNA following transfection into Lec23 cells. Surprisingly, however, when cDNAs carrying these mutations were introduced into Lec23 cells, a significant level of L-PHA binding was restored (Fig. 4B). Thus α-glucosidase I with either of the known CDG IIb mutations was able to rescue the phenotype of Lec23 almost as well as wild-type enzyme. Nevertheless, when cell extracts were prepared from stable transfectants expressing the same mutant cDNAs, their α-glucosidase I activity was extremely low in vitro (Table II) as reported previously (23). Thus human GCS1 with the mutation R486T or F652L has good α-glucosidase I activity in vivo in the Lec23 CHO cellular environment but almost none in vitro, whereas GCS1 with the S440F mutation has no α-glucosidase I activity in vitro and also very little in vivo (10) (Fig. 2). However, we discovered that a few complex N-glycans are synthesized by Lec23 cells and can be readily detected on N-CAM.

**Lec23 Cells Express PSA on N-CAM**—The MALDI-TOF mass spectrometric analyses of the N-glycans on Lec23 glycoproteins identified small amounts of complex N-glycans (Fig. 2A) as was observed previously on vesicular stomatitis virus G glycoprotein from Lec23 cells (10). The synthesis of complex N-glycans by Lec23 cells was also revealed by investigating the presence of PSA on N-CAM by Western blot analysis. PSA is a polymer of (2,8)-linked sialic acids attached to the terminal sialic acid of complex N-glycans in mammals (reviewed in Ref. 42). Previous experiments have shown that N-CAM is essentially the only glycoprotein in CHO cells that can be detected with anti-PSA antibodies (43, 44).

To determine whether Lec23 cells make PSA on N-CAM, proteins extracted in 1% Nonidet P-40 and subjected to Western blot analysis with anti-FLAG antibody. B, the same cells were incubated with FITC-labeled L-PHA and analyzed by FACS. Bold lines, transfected Lec23 cells; thin lines, non-transfected Lec23 cells; dotted lines, non-transfected CHO cells.

**TABLE II**

| Transfected cDNA | α-Glucosidase I | β4GalT-1 |
|------------------|----------------|----------|
|                  | cpm/mg/h       | nmol/mg/h|
| Vector           | 37             | ND       |
| GCS1             | 4593           | 22.6     |
| GCS1/S440F       | 90             | 28.2     |
| GCS1/R486T       | 76             | 29.9     |
| GCS1/F652L       | 131            | 25.1     |

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both PNGase F and Endo H, as expected if most N-glycans were oligomannosyl.

When this blot was stripped and reprobed with anti-PSA mAb 735, it could be seen that only a small fraction of N-CAM in CHO cells was modified by PSA. This species of N-CAM of ~240 kDa was sensitive to digestion with PNGase F but not to Endo H in both CHO and Lec23 extracts. Therefore, the PSA in Lec23 N-CAM was attached to complex N-glycans. Interestingly, the amount of Lec23 PSA-N-CAM was similar to that in parent CHO cells, suggesting that the few complex N-glycans synthesized in Lec23 were disproportionately expressed on N-CAM, perhaps for the purpose of making PSA-N-CAM. A similar result was obtained by FACS analysis (Fig. 5), although in this case, CHO cells clearly bound more anti-PSA mAb than Lec23 cells. Lec2 CHO cells that are deficient in CMP-sialic acid transport expressed almost no PSA as shown previously (27, 45, 46).

**DISCUSSION**

Here we identify the molecular basis of the lec23 mutation in CHO cells as a missense mutation in the Gcs1 gene coding region. Lec23 cells express only the mutant Gcs1 gene. The single base change that converts Ser to Phe occurs in a highly conserved region of exon 4. When the analogous mutation

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**FIG. 5.** Lec23 N-CAM is modified with PSA on complex N-glycans. A, proteins were extracted in 1.5% Nonidet P-40 from 2 x 10⁶ cells and mixed with bovine pancreatic RNase B before treatment with PNGase F or Endo H for 5 h. Proteins were separated in a 6% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. N-CAM was detected with the anti-N-CAM antibody NCAM13. PSA-modified N-CAM proteins were detected by reprobing with anti-PSA mAb 735. RNase B was stained with Coomassie Blue after analysis of the same reactions on a 20% gel. B, Lec2, Lec23, and CHO cells were analyzed by FACS with the anti-N-CAM antibody NCAM13 or anti-PSA mAb 735 and FITC-conjugated anti-mouse IgG+M antibody. **Bold lines**, anti-N-CAM or anti-PSA antibodies with FITC-conjugated anti-mouse IgG+M antibody; **thin lines**, FITC-conjugated anti-mouse IgG+M antibody alone.
S440F was engineered into a full-length human GCS1 cDNA, the mutant cDNA was unable to rescue the Lec23 phenotype, and transfectants had almost no detectable α-glucosidase I activity in vitro despite overexpression of the cDNA. The Lec23 mutant is therefore useful for studying the properties of mutant GCS1 genes, for investigating alternative pathways of N-linked glycosylation, and for identifying the consequences to the cell of maintaining Glc residues on glycoproteins and oligosaccharides that exit the endoplasmic reticulum and traffic through the cell.

Previous studies of α-glucosidase I from rat mammary gland have shown that it is an ~85-kDa glycoprotein with a single transmembrane domain and a luminally oriented catalytic domain (40). A sulphydryl group has been identified in the active site (47), and the C-terminal 39 kDa of the enzyme contains the catalytic activity (40, 41). The expression level of α-glucosidase I is regulated during lactation and by hormone treatments (39). The S440F mutation identified in Lec23 cells is a novel α-glucosidase I-inactivating mutation. The only other GCS1 mutations were identified in a CDG IIb patient (23). This patient was a compound heterozygote and carried both R486T and S440F mutations. The S440F mutation identified in Lec23 cells is a novel activity may be responsible for removing the terminal Glc from Lec23 glycoproteins, thereby generating its physiological substrate and allowing all three Glc residues to be removed. Alternatively, a novel activity may be responsible for removing the terminal Glc of Man$_p$GlcNAc$_2$ in the absence of α-glucosidase I.

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