Identification of Specific Glycoforms of Major Histocompatibility Complex Class I Heavy Chains Suggests That Class I Peptide Loading Is an Adaptation of the Quality Control Pathway Involving Calreticulin and ERp57*

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Glycosylation analysis was used to probe the sequence of events accompanying the binding of antigenic peptides to the major histocompatibility complex class I heavy chains. Free heavy chains were isolated from the β2-microglobulin-negative cell line Daudi and from the B-lymphoblastoid cell line Raji. Heavy chains were also isolated from Raji cells in multimolecular complexes (peptide loading complexes) containing the transporter associated with antigen processing, tapasin and ERp57 (peptide loading complexes) containing the transporter associated with antigen processing, tapasin and ERp57, allowing glycan maturation and transport of the assembled class I-β2m dimer, together with associated CRT and ERp57, is associated with a larger complex that also contains the TAP heterodimer and the transmembrane glycoprotein tapasin (10). Tapasin provides the bridge that links MHC class I to the TAP heterodimer (5), and a disulfide bond between tapasin and ERp57 is dependent upon the association of class I molecules with the complex (9). Peptides destined for MHC class I binding are translocated in an ATP-dependent fashion into the ER by the TAP heterodimer. Here they may undergo further N-terminal trimming prior to binding to TAP-associated MHC class I molecules (11). Peptide binding induces dissociation of the class I-β2m dimer from the tapasin-TAP complex, as well as from CRT and ERp57, allowing glycan maturation and transport of the assembled class MHC class I-β2m peptide complex from the ER.

CNX and CRT are both lectin-like molecules that can facilitate the folding of newly synthesized glycoproteins in the ER, in part by binding to their N-linked glycans (12, 13). In the early stages of oligosaccharide processing, HCs, in common with all nascent glycoproteins, transiently carry the Glc1Man9GlcNAc2 oligosaccharide (Fig. 1). The α1,3 arm of this glycan, in particular the terminal Glc residue (G3), provides the ligand that enables the partially folded protein to interact with the lectin-binding sites on both CNX (12) and CRT (13). A quality control cycle has been proposed in which CNX and CRT in turn recruit ERp57, which facilitates the proper formation of disulfide bonds in the nascent glycoprotein (14). Removal of the glucose residue by glucosidase II eliminates CNX or CRT binding. Only

In the endoplasmic reticulum (ER), 1 major histocompatibility complex (MHC) Class I molecules bind short peptides that are generated in the cytosol by proteasomal degradation and translocated into the ER by the transporter associated with antigen processing (TAP). The class I-peptide complexes are then transported to the cell surface where they can be screened by CD8-positive T cells and can potentially trigger an immune response (1). The detailed mechanisms that regulate peptide binding to MHC class I molecules are not yet fully established.

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1 The abbreviations used are: ER, endoplasmic reticulum; β2m, β2 microglobulin; CNX, calnexin; CRT, calreticulin; Gu, glucose unit; HC, heavy chain; MALDI, matrix-assisted laser desorption/ionization; MHC, major histocompatibility complex; HPLC, high-performance liquid chromatography; TAP, transporter associated with antigen processing.

Mature MHC class I molecules consist of two subunits: the heavy chain (HC), which is a transmembrane glycoprotein, and a small, soluble, nonglycosylated protein, β2 microglobulin (β2m). A peptide must associate with the peptide-binding groove formed by the α1 and α2 domains of the HC for proper folding of the class I-β2m dimer and its subsequent transport to the cell surface. Class I assembly requires multiple coordinated intra- and inter-molecular events to ensure the continuous reporting of cellular contents to cytotoxic T-lymphocytes.

It has been proposed that in the ER of humans, unassembled heavy chains interact initially with the membrane-bound chaperone, calnexin (CNX) (2, 3), or with the soluble chaperone BiP (4). Concomitant with β2m association the heavy chain is released and binds to the soluble chaperone, calreticulin (CRT) (5). Either CNX or CRT recruits the thiol oxidoreductase ERp57 into the complex (6–8). This appears to facilitate the formation and maintenance of an intrachain disulfide bond in the class I heavy chain, most likely that which anchors the α2 domain α-helix to the floor of the peptide-binding groove (9). The assembled class I-β2m dimer, together with associated CRT and ERp57, is associated with a larger complex that also contains the TAP heterodimer and the transmembrane glycoprotein tapasin (10).

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Peptides destined for MHC class I binding are translocated in an ATP-dependent fashion into the ER by the TAP heterodimer. Here they may undergo further N-terminal trimming prior to binding to TAP-associated MHC class I molecules (11). Peptide binding induces dissociation of the class I-β2m dimer from the tapasin-TAP complex, as well as from CRT and ERp57, allowing glycan maturation and transport of the assembled class MHC class I-β2m peptide complex from the ER.

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MHC I Peptide Loading Uses the Calreticulin/ERp57 Pathway

**Sample Preparation**—Raji wild type B cells or Daudi β₂m-negative B cells (10^10) were lysed in 200 ml of 1% digitonin in 0.15 M NaCl, 0.01 M Tris, pH 7.4 (Tris-buffered saline) containing protease inhibitors as previously described (10) and passed through one of three affinity columns consisting of MaP.ERp57 (a-ERp57), 148.3 (α-TAP), or HC10 (α-HC) coupled to BioGel A15m beads (Bio-Rad) to produce ERp57-associated proteins, TAP-associated proteins, or free class I HC, respectively. After extensive washing with 0.1% digitonin in Tris-buffered saline, the proteins were eluted in 1% octylglucoside in 5.5 mM MgCl₂, and dialyzed against 0.1% octylglucoside in phosphate-buffered saline. During dialysis some protein precipitated that was removed by centrifugation. These preparations produced the following samples: (i) from the α-ERp57 affinity column: ERp57/CRT, ERp57-associated tapasin and ERp57-associated class I HC in solution (gel A), and ERp57-associated class I HC as a precipitate (gel B); (ii) from the α-TAP affinity column: ERp57/CRT, TAP-associated tapasin and TAP-associated class I HC in solution (gel C), and TAP-associated class I HC as a precipitate (gel D); (iii) from the α-HC affinity column: free class I HC from Raji cells (gel E) and Daudi cells (gel F); and (iv) from the α-TAP affinity column: tapasin from Daudi cells (gel G) (Fig. 2). Precipitated samples were dissolved with sonoication in 2–10% SDS.

**SDS-PAGE Gels**—Samples of each of the proteins were run on 10% SDS-PAGE gels in a vertical mini-gel system, 80 × 80 × 0.75 mm at 500 V and 25 mA/gel. The gels were prepared according to Kuster et al. (23), and 20 μl of sample and 5 μl of 5× Laemmli sample buffer were used per gel. Reduction and alkylation of the proteins were carried out prior to running the gels. Dithiothreitol (10 mM, 0.5 μl) was added, and each sample was incubated at 70 °C for 10 min. Iodoacetamide (100 mM) was added to a final concentration of 10 mM, and the samples were incubated for 30 min at room temperature in the dark. Up to 8 wells of each sample were run to provide sufficient gels for NP-HPLC analysis, enzyme digestions, and mass spectrometry. The protein was visualized in the gel by staining with Coomassie Blue for at least 2 h, destaining with 50% methanol/7% acetic acid, and then overnight with 5% methanol/7% acetic acid. The bands were excised, cut into pieces of ~1 mm², and frozen at ~20 °C for at least 2 h. The gel pieces were washed with 2 × 300 μl of 20 mM NaHCO₃, pH 7.0, for 30 min and then with 300 μl of 1:1 acetonitrile–20 mM NaHCO₃ for 60 min to remove residual SDS (23). The gel pieces were dried in a vacuum centrifuge.

**In Situ Digestion of N-Glycans with Peptide N-Glycanase F and Glycan Extraction**—The volumes given are for 10–15 mm² of SDS-PAGE gel band containing the protein of interest, 30 μl of peptide N-glycanase F (100 units/ml) were added to the gel pieces and left for about 5 min for the gel to re-swell, and then the gel was covered with 20 mM NaHCO₃. The sample was incubated at 37 °C for 16 h. After incubation, the sample was centrifuged at 1,100 × g for 5 min. The supernatant was retained, and the glycans were extracted from the gel using three changes of 200 μl of sub-boiling point distilled water and sonicated for 30 min, followed by extractions with 200 μl of acetonitrile, 200 μl of water, and 200 μl of acetone. The extracts and the above supernatant were combined. 30 μl of activated anion exchange resin, AG-50 X12 (H⁺) slurries were added to the extracts and incubated for 5 min at room temperature to desalt. It was centrifuged for 5 min at 550 × g, and the supernatant was filtered with a 0.45-μm Millex-LH/ hydrophilic polytetrafluoroethylene filter attached to a 2.5-ml syringe. The supernatant was partially dried in a vacuum centrifuge, transferred to a 0.5-ml microcentrifuge tube, and dried completely.

**Fluorescent Labeling and N-Peptide HPCL**—The samples were labeled with 2-aminobenzamide by reductive amination according to Bigge et al. (24) and processed through NP-HPLC using the low salt buffer system as...
described by Guile et al. (25). This uses a 4.6 × 250-mm GlycoSep-N column (Glyko) with a gradient of 20–58% solvent A (solvent A, 50 mM formic acid adjusted to pH 4.4 with ammonia; solvent B, acetonitrile). Each 2-aminobenzamide-labeled sample was dissolved in 100 μl of sub-boiling point distilled water, and 20 μl was run on the NP-HPLC with 80 μl of acetonitrile. The system was calibrated using an external standard of hydrolyzed and 2-aminobenzamide-labeled glucose oligomers to create a dextran ladder (25). The number of glucose residues in each dextran peak was plotted against the retention times of the peaks to obtain a standard curve. The retention times for the individual glycans were converted to glucose units (GU) using this curve. The retention times were then compared with a data base of experimental values. The higher the GU value the larger the glycan it represents. The incremental GU values are measures of the affinity of each glycan for the column matrix and are related to the hydrophilicity of each monosaccharide residue in the chain. It also depends on how much of the hydrophilic surface is exposed to the column matrix. In the case of GlcNAc, the N-acetyl side chain hinders some of the interactions which are available to glucose; therefore the incremental value for GlcNAc is about 50% of that of glucose (25). It is this feature that gives the fine specificity of the columns, allowing the distinction between arm-specific isomers. The area of each

![Fig. 3. SDS-PAGE gels of complexes from Raji cells eluted from the α-ERp57 (A and B) and the α-TAP (C and D) affinity columns. The bands containing ERp57/CRT, HC, and tapasin are indicated. The NP-HPLC chromatograms (AHC, BHC, CHC, and DHC) are of the glycans released from the CRT associated HCs in gels A, B, C, and D, respectively. Each of these glycan profiles contains predominantly Man,GlcNAc (peak 4) and Glc,Man,GlcNAc (peak 5).](http://www.jbc.org/content/392/21/5964/F3.large.jpg)

![Fig. 4. SDS-PAGE gels of complexes eluted from the α-HC affinity column from Raji cells (E) and Daudi cells (F). The NP-HPLC chromatograms (EHC and FHC) are of the glycans released from free HC, which were not associated with CRT, in gels E and F, respectively. The glycan profiles of E and F contain no Glc,Man,GlcNAc, but more of the smaller glycans, Man,GlcNAc, and Man,GlcNAc.](http://www.jbc.org/content/392/21/5964/F4.large.jpg)

![Fig. 5. SDS-PAGE gels of Raji cell complexes eluted from the α-ERp57 affinity column (A) and from the α-TAP affinity column (C) and of a Daudi cell complex also eluted from the α-TAP affinity column (G). The NP-HPLC chromatograms (A TPN, C TPN, and G TPN) are of the glycans released from the tapasin bands in gels A, C, and G, respectively. The tapasin bands contain mainly Man,GlcNAc and a small peak of Glc,Man,GlcNAc.](http://www.jbc.org/content/392/21/5964/F5.large.jpg)
of the glycans peaks was measured and expressed as a percentage of the total glycans of each sample. Comparing the percentage areas of intact and digested glycans pools helps to identify glycans that co-elute.

**Enzyme Digestions**—Approximately 100–300 fmol of each glycans pool were dried, and 10 μl of 50 units/ml Jack bean α-mannosidase in 10 mM sodium acetate with 2 mM Zn²⁺, pH 5, were added. The reaction mixture was incubated at 37 °C overnight, after which a further 10 μl of 100 units/ml enzyme were added, and the sample was incubated for a further 24 h. The sample was then heated at 100 °C for 5 min to denature the enzyme and dried in a vacuum centrifuge. It was redisolved in 20 μl of sub-boiling point distilled water and analyzed by NP-HPLC. Similarly two additions of 10 μl of α1,3-glucosidase II (in 50 mM triethylamine, pH 7, 0.1 M sodium chloride, 10% glycerol and sodium azide) were made to 100–300 fmol of the released glycans. The samples were processed as for the mannose digests. The NP-HPLC profiles were analyzed according to Guile et al. (25).

**Matrix-assisted Laser Desorption Ionization Time-of-flight Mass Spectrometry**—Positive ion Reflectron MALDI time-of-flight mass spectra were recorded with a Micromass ToFSpec 2E Reflectron mass spectrometer (Micromass Ltd., Manchester, UK) fitted with delayed extraction and a nitrogen laser (337 nm). The acceleration voltage was 20 kV, and the delay for the delayed extraction ion source was 500 ns. The samples were prepared by adding 0.5 μl of an aqueous solution of the sample to the matrix solution (0.5 μl of a saturated solution of 2,5-dihydroxybenzoic acid in acetonitrile) on the stainless steel target plate, allowing it to dry at room temperature and then recrystallizing it from ethanol.

**RESULTS**

**Isolation of Glycans**—Heavy chain complexes, free HC, and tapasin (Figs. 3–5) were produced by passing lysed Raji wild type B-cells or Daudi β₂m-negative B cells through one of three affinity columns, α-ERp57, α-TAP, or α-HC (Fig. 2). The glycosylation of the following proteins was analyzed: ERp57/CRT, ERp57-associated tapasin and ERp57-associated class I HC (gel A; Figs. 3 and 5); ERp57-associated class I HC (gel B; Fig. 3); TAP-associated tapasin and TAP-associated class I HC (gel C; Figs. 3 and 5); TAP-associated class I HC (gel D; Fig. 3); class I HC from Raji cells (gel E; Fig. 4); class I HC from Daudi cells (gel F; Fig. 4); and tapasin from Daudi cells (gel G; Fig. 4). The protein bands on the SDS-PAGE gels (Figs. 3–5) were identified according to their migration positions and by Western blotting. The approximate apparent molecular masses were as follows: ERp57/calreticulin, 55 kDa; tapasin, 50 kDa; and HC, 45 kDa. The tapasin bands were confirmed by N-terminal sequencing, which indicated that the Raji cell tapasin ran as a doublet (Figs. 3 and 5). Fig. 6 shows Western blotting performed on a selection of samples. The upper panel shows the reactivity of the samples with the tapasin C-terminal specific rabbit antiserum Rgp48C, and the lower panel shows the reactivity of the samples with the anti-HC-terminal peptide-specific rabbit antiserum RA3e7. The specificity of both the tapasin-reactive and the HC-reactive antibodies is demonstrated by samples G (tapasin from Daudi cells) and E (free HC purified from Raji cells). Samples A–C all contain tapasin and HC. Samples A and C contain tapasin and HC in solution, and sample B has some tapasin co-precipitating with HC during dialysis.

Glycans were released by incubating the excised gel bands with peptide N-glycanase F, which cleaves the N-glycosidic linkage to asparagine, and the glycan pools labeled with the fluorophore 2-aminobenzamide. The chromatograms of both the HC and the tapasin samples were all found to contain the same sugars in the peaks, labeled from 1 to 5, although in different proportions (Figs. 3–5). Peak a (Figs. 3–5) was not identified. It is present in the samples eluted from the α-ERp57 and α-HC columns but not in those eluted from the α-TAP column (gels C and D), and it is not digested by either mannosidase or glucosidase II and was not seen in the MALDI mass spectrometric analysis (see Table III). As expected, the ERp57/CRT contained no glycans that could be detected by NP-HPLC because ERp57 and human CRT are not glycosylated (data not shown). In every case, glycan structures were assigned from the GU values of the peaks in the NP-HPLC trace of the intact glycan pools (Table I) combined with data from glucosidase II and Jack bean α-mannosidase digests including consideration of peak areas (Table II). The assignments are consistent with MALDI mass spectrometric data (Table III).

**Glucosidase II Digestions**—The identity of the peaks in the chromatograms in Figs. 3–5 was confirmed with glucosidase II and Jack bean α-mannosidase digests. Glucosidase II cleaves the nonreducing terminal glucose α1–3-mannose linkage. Fig. 7 shows the glucosidase II digest of ERp57 associated class I HC (B_HC), in which peak 5 (GU 10.15) is completely digested to Man₉GlcNAc₂, peak 4 (GU 9.49) (Table I). The incremental GU value for one glucose monomer is 0.7, so the digestion of peak 5 to peak 4 with a difference of −0.7 and an increase in peak area of peak 4 from 37.4 to 61.8% confirms the presence of Glc₃Man₉GlcNAc₂ in peak 5. Peak 5 was collected and digested with glucosidase II producing a single peak of Man₆GlcNAc₂ (Fig. 7, inset a), confirming that peak 5 contains only Glc₃Man₉GlcNAc₂. A comparison of the percentage peak areas of the intact glycan pool of the ERp57 associated class I HC (B_HC) with that of its glucosidase II digest shows the

![Table I](https://www.jbc.org/doi/10.1074/jbc.M112.402995)

**GU values and peak identification of the glycans of the heavy chain and tapasin samples**

| Peak number | Identification | Gel A (α-ERp57 free HC from solution) | Gel B (α-ERp57 free HC from precipitate) | Gel C (α-TAP free HC from solution) | Gel D (α-TAP free HC from precipitate) | Gel E (α-HC from solution) | Gel F (Daudi HC) | Gel A (α-ERp57 tapasin) | Gel B (α-TAP tapasin) | Gel G (Daudi tapasin) |
|-------------|----------------|-------------------------------------|---------------------------------------|------------------------------------|--------------------------------------|--------------------------|-----------------|----------------------|----------------------|----------------------|
| 1           | Man₉GlcNAc₂   | 7.06                                | 7.04                                  | 7.07                               | 7.04                                 | 7.05                     | 7.05            | 7.05                 | 7.05                 | 7.05                 |
| 2           | Man₉GlcNAc₂   | 7.93                                | 7.93                                  | 7.93                               | 7.93                                 | 7.93                     | 7.93            | 7.91                 | 7.95                 | 7.95                 |
| 3a          | Man₉GlcNAc₂   | 8.6                                 | 8.61                                  | 8.7                                | 8.60                                 | 8.71                     | 8.71            | 8.58                 | 8.7                  | 8.67                 |
| 3b          | Man₉GlcNAc₂   | 8.81                                | 8.79                                  | 8.80                               | 8.80                                 | 8.82                     | 8.82            | 8.79                 | 8.82                 | 8.81                 |
| a           | (artifact)    | 9.5                                 |                                       |                                     |                                      | 9.34                     | 9.34            | 9.3                  | 9.3                  | 9.3                  |
| 4           | Glc₃Man₉GlcNAc₂ | 9.5                                | 9.49                                  | 9.5                                | 9.49                                 | 9.5                      | 9.5             | 9.5                  | 9.5                  | 9.5                  |
| 5           | Glc₃Man₉GlcNAc₂ | 10.16                             | 10.15                                 | 10.18                               | 10.16                                | 10.15                    | 10.15           | 10.17                | 10.18                | 10.18                |

* Isoforms.
The results of the carbohydrate analysis undertaken here are consistent with the hypothesis that the glucosylated glyco-
forms (Glc3Man7GlcNAc2) of MHC class I bind to CRT. In an adaptation of the CNX/CRT quality control pathway, we propose that the association of a peptide with the binding groove of MHC class I indicates that the folding/assembly process is complete. At this stage in the CRT quality control pathway, the final glucose residue is removed permanently by glucosidase II because the UDP-glucosyl transferase does not reglucosylate fully folded glycoproteins. Therefore the removal of the glucose residue allows the class I-peptide complex to leave the quality control pathway and to dissociate from the other components of the loading complex (Fig. 10). The TAP- and ERp57-associated HCs that are not monoglucosylated (50% of the total) may correspond to class I-H2m dimers that have been deglucosylated but have not yet dissociated from the loading complex. At first sight these data appear to be inconsistent with previous studies indicating that the ratio of CRT to HCs in the loading complex was 0.9 to 1 (10). This ratio might be used to argue that all of the HC in the loading complex should contain a monoglucosylated glycan. However, the amounts of monoglucosylated HC and CRT may in fact be quite similar. Whether or not the deglucosylated class I molecules in the loading complex contain bound peptides is an interesting question for future studies. In addition, these data could also be consistent with a glycan-independent interaction between CRT and HC.

Other interpretations of these data include the possibility that the MannGlcNAc2 sugar attached to the HC still retains sufficient binding affinity for CRT for the glycoforms to remain associated with the loading complex during purification. The data would also be consistent with the proposal that unfolded glycoproteins interact with both a lectin site and a nonspecific polypeptide-binding site in CNX and CRT (29–31).

Fig. 7. NP-HPLC chromatogram of the glycans released from the SDS gel band containing HC (BHC) eluted in a complex from Raji cells that bound to the α-ERp57 affinity column (see Fig. 3). The lower chromatogram shows the glucosidase II digest of the same sample. Glc3Man3GlcNAc2 (of peak 4) and Glc3Man4GlcNAc2 (peak 5) digest to Man3GlcNAc2 and Man4GlcNAc2, respectively. Inset a is of an individual fraction of Glc3Man3GlcNAc2 (peak 5) and the product of glucosidase II digestion, a single peak of Man3GlcNAc2. Inset b is of an individual fraction of Man4GlcNAc2 and Glc3Man4GlcNAc2 (peak 4) and the product of glucosidase II digestion, Man3GlcNAc2 together with Man4GlcNAc2. Molecular representations of some of the glycoforms are included. The key below the figure shows the monosaccharides, which are presented schematically. The linkage positions of the oligosaccharides are represented by the angle of the line linking adjacent monosaccharides. The number indicates the position of the ring carbon in the sugar on the right that attaches to the sugar on the left. In this paper the sugar on the left is always linked via the C1 reducing end.

Fig. 8. NP-HPLC chromatogram of the glycans released from the SDS gel band containing HC (BHC) eluted in a complex from Raji cells that bound to the α-ERp57 affinity column (see Fig. 3). The lower chromatogram shows the Jack bean α-mannosidase digest of the same sample. Glc3Man3GlcNAc2 (peak 4) and Glc3Man4GlcNAc2 (peak 5) were digested to Glc3Man3GlcNAc2 (peak 7) and Glc3Man4GlcNAc2 (peak 8). Man5GlcNAc2 (peaks 1–4) were digested to Man5GlcNAc2 (peak 6). Molecular representations of some of the glycoforms are included. For key see Fig. 7.
50% of the HC glycoforms contain Glc1Man9GlcNAc2 and interact non-covalently with CRT independently of the glycosylation through protein-protein interactions (31). Alternatively these HCs may associate with CRT through the oligosaccharide structure. The remaining 50% contain Man6GlcNAc2 glycan pools and the glucosidase II digest were compared (Table IV). The small fraction of the TAP- and ERp57-associated molecules with the TAP complex are mutually exclusive (20). It was suggested that the CNX-associated species might represent a glycan-independent component, as recently suggested by Danilczyk and Williams (38).

**Glycosylation of Tapasin**—Tapasin glycosylation is significantly different from the class I HC’s (Figs. 3–5 and Table IV). Tapasin co-purifying with ERp57 or TAP contained no detectable Glc1ManN4GlcNAc2, when the peak areas of the intact glycan pool and the glucosidase II digest were compared (Table II), and less than 10% of the glycan has the Glc1Man6GlcNAc2 structure. 40–60% of the tapasin glycans are Man6GlcNAc2 (Table IV). The small fraction of the TAP- and ERp57-associated tapasin molecules containing Glc1Man6GlcNAc2 may be folding intermediates. Previously it was established that a subset of tapasin-TAP complexes are associated with CNX and ERp57 and that the interaction of CNX and MHC class I molecules with the TAP complex are mutually exclusive (20). It was suggested that the CNX-associated species might represent tapasin in the process of folding. The CNX-tapasin interaction can be seen even with truncated tapasin molecules that lack the N-linked glycan, but nevertheless the presence of the monoglucosylated glycoform in a subset of the TAP-associated tapasin species may indicate that the subset is associated with CNX and ERp57 via the lectin site on CNX. The predominance of Man6GlcNAc2 glycans in the tapasin pool probably reflects the fact that tapasin, which is properly folded and retained in...
the ER by a specific retention signal in its cytoplasmic domain (10), contains glycans that are somewhat inaccessible to the ER mannosidases.

**Conclusion**—In conclusion, this paper demonstrates that ~50% of the sugars N-linked to class I heavy chains in association with CRT and ERp57 are monoglucosylated. This is consistent with the class I peptide loading process being an adaptation of the general quality control mechanism involving CRT and ERp57.

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**REFERENCES**

1. Pamer, E., and Cresswell, P. (1998) *Ann. Rev. Immunol.* **16**, 323–358
2. Degen, E., Cohen-Doyle, M. F., and Williams, D. B. (1992) *J. Exp. Med.* **175**, 1653–1661
3. Furnery, M. R., Allen, S., Allen, A. J., and Bulleid, N. J. (2000) *J. Biol. Chem.* **275**, 14933–14938
4. Nosser, E., and Parham, P. (1995) *J. Exp. Med.* **181**, 327–337
5. Sadasivan, B., Lehner, P. J., Ortmann, B., Spies, T., and Cresswell, P. (1996) *Immunity* **5**, 103–114
6. Lindquist, J. A., Jensen, O. N., Mann, M., and Hammerling, G. J. (1998) *Eur. Mol. Biol. Organisation J.* **17**, 2186–2195
7. Hughes, E. A., and Cresswell, P. (1998) *Curr. Biol.* **8**, 709–712
8. Morrice, N. A., and Powis, S. J. (1998) *Curr. Biol.* **8**, 713–716
9. Dick, T. P., Bangia, N., Peaper, D. R., and Cresswell, P. (2002) *Immunity* **16**, 87–98
10. Ortmann, B., Copeman, J., Lehner, P. J., Sadasivan, B., Herberg, J. A., Grandea, A. G., Riddell, S. R., Tampe, R., Spies, T., Trowsdale, J., and Cresswell, P. (1997) *Science* **277**, 1306–1309
11. Paz, P., Brouwerstijn, N., Perry, R., and Shastr, N. (1999) *Immunity* **11**, 241–251
12. Bergeron, J. J., Zapun, A., Ou, W. J., Hemming, R., Parlati, F., Cameron, P. H., and Thomas, D. Y. (1998) *Adv. Exp. Med. Biol.* **435**, 105–116
13. Hellen, A., and Abi, M. (2001) *Science* **291**, 2364–2369
14. Oliver, J. D., Roderick, H. L., Llewellyn, D. H., and High, S. (1999) *Mol. Biol. Cell* **10**, 2573–2582
15. Liu, Y., Choudhury, P., Cabral, C. M., and Sifers, R. N. (1999) *J. Biol. Chem.* **274**, 3661–3667
16. Suh, W. K., Mitchell, E. K., Yang, Y., Peterson, P. A., Waneck, G. L., and Williams, D. B. (1996) *J. Exp. Med.* **184**, 337–348
17. Vassilakos, A., Cohen-Doyle, M. F., Potter, P. A., Jackson, M. R., and Williams, D. B. (1996) *EMBO J.* **15**, 1495–1506
18. Jackson, M. R., Cohen-Doyle, M. F., Peterson, P. A., and Williams, D. B. (1994) *Science* **263**, 384–387
19. Margolese, L., Waneck, G. L., Suzuki, C. K., Degen, E., Flavell, R. A., and Williams, D. B. (1993) *J. Biol. Chem.* **268**, 17959–17966
20. Diedrich, G., Bangia, N., Pan, M., and Cresswell, P. (2001) *J. Immunol.* **166**, 1703–1709
21. Meyer, T. H., van Endert, P. M., Uebel, S., Ehring, B., and Tampe, R. (1994) *FEBS Lett.* **351**, 443–447
22. Karlsson, G. B., Butters, T. D., Dwek, R. A., and Platt, F. M. (1993) *J. Biol. Chem.* **268**, 570–576
23. Kuster, B., Wheeler, S. F., Hunter, A. P., Dwek, R. A., and Harvey, D. J. (1997) *Anal. Biochem.* **250**, 82–101
24. Bigge, J. C., Patel, T. P., Bruce, J. A., Goulding, P. N., Charles, S. M., and Parekh, R. B. (1995) *Anal. Biochem.* **230**, 229–238
25. Guile, G. R., Rudd, P. M., Wing, D. R., Prime, S. B., and Dwek, R. A. (1996) *Anal. Biochem.* **240**, 210–226
26. Patel, A. R., Thomas, C. J., and Suresh, A. (2000) *J. Biol. Chem.* **275**, 24348–24356
27. Harris, M. R., Yu, Y. Y., Kindle, C. S., Hansen, T. H., and Solomon, J. C. (1998) *J. Immunol.* **160**, 5404–5409
28. Lehner, P. J., Surman, M. J., and Cresswell, P. (1998) *Immunity* **8**, 221–231
29. Ware, F. E., Vassilakos, A., Peterson, P. A., Jackson, M. R., Lehrman, M. A., and Cresswell, P. (1997) *Science* **277**, 1306–1309

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and Williams, D. B. (1995) *J. Biol. Chem.* **270**, 4697–4704
30. Ihara, Y., Cohen-Doyle, M. F., Saito, Y., and Williams, D. B. (1999) *Mol. Cell.* **4**, 331–341
31. Leach, M. R., Cohen-Doyle, M. F., Thomas, D. Y., and Williams, D. B. (2002) *J. Biol. Chem.* **277**, 29686–29697
32. Barber, L. D., Patel, T. P., Percival, L., Gumperz, J. E., Lanier, L. L., Phillips, J. H., Bigge, J. C., Wormwald, M. R., Parekh, R. B., and Parham, P. (1996) *J. Immunol.* **156**, 3275–3284
33. Kornfeld, R., and Kornfeld, S. (1985) *Annu. Rev. Biochem.* **54**, 631–664
34. Jakob, C. A., Burda, P., Roth, J., and Aebl, M. (1998) *J. Cell Biol.* **142**, 1223–1233
35. Ellgaard, L., Molinari, M., and Helenius, A. (1999) *Science* **286**, 1882–1888
36. Schumacher, T. N., Heemels, M. T., Neefjes, J. J., Kast, W. M., Melief, C. J., and Ploegh, H. L. (1990) *Cell* **62**, 563–567
37. Baas, E. J., van Santen, H. M., Kleijmeer, M. J., Geuze, H. J., Peters, P. J., and Ploegh, H. L. (1992) *J. Exp. Med.* **176**, 147–156
38. Danicczyk, U. G., and Williams, D. B. (2001) *J. Biol. Chem.* **276**, 25532–25540
Identification of Specific Glycoforms of Major Histocompatibility Complex Class I Heavy Chains Suggests That Class I Peptide Loading Is an Adaptation of the Quality Control Pathway Involving Calreticulin and ERp57

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