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J. Biochem. Biophys. Methods 62 (2005) 1–12
JOURNAL OF biochemical and biophysical methods
www.elsevier.com/locate/jbbm

Reconstitution of glycopeptide export in mixed detergent-solubilised and resealed microsomes depleted of lumenal components

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Received 1 July 2003; received in revised form 22 December 2003; accepted 1 January 2004

Abstract

Export of macromolecules from the endoplasmic reticulum (ER) lumen into the cytosol is a major aspect of the quality control systems operating within the early secretory system. Glycopeptides are exported from the ER by an ATP- and GTP-dependent pathway, which shares many similarities to the protein export system. Significantly, for glycopeptides, there is no requirement for cytosolic factors, biochemically distinguishing the glycopeptide and protein paths and probably reflecting the lower conformational complexity of the former substrate. Genetic studies in yeast, and biochemical data from higher eukaryotes, indicate that glycopeptides utilise the Sec61 translocon. Here, we report a new system allowing access to lumenal ER components, facilitating assessment of their importance in glycopeptide retrotranslocation and potentially other processes. Saponin, in combination with CHAPS, but not saponin alone, facilitated removal of >95% of lumenal protein disulphide isomerase (PDI) and BiP. Upon resealing, these microsomes retained glycopeptide export competence. These

Abbreviations: ER, endoplasmic reticulum; ConA, concanavalin A; PMSF, phenylmethylsulphonyl fluoride; PDI, protein disulphide isomerase; SRPR, signal recognition particle receptor; RI, ribophorin I.

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0165-022X/$ - see front matter
doi:10.1016/j.jbbm.2004.01.013

data suggest that the majority of lumenal components of the ER are most likely nonessential for glycopeptide export. In addition, export competence was highly sensitive to the addition of external protease, indicating a role for protein factors with cytoplasmically exposed determinants.

Keywords: Retrograde transport; Endoplasmic reticulum; Quality control; Glycopeptide; Reconstitution

1. Introduction

The translocon responsible for signal sequence-mediated import of proteins into the lumen of the endoplasmic reticulum (ER) is a multiprotein complex, of which Sec61 is the major component contacting the actively translocating polypeptide chain and maintaining it in an aqueous environment during passage across the ER lipid bilayer [1–3]. During translocation various processes, including removal of the signal peptide, disulphide bond formation and posttranslational modifications, principally glycosylation, are initiated and the protein enters folding pathways and assembly into complexes assisted by molecular chaperones present in the ER lumen [4–6]. Only fully folded and assembled proteins are allowed to proceed to their final destination, normally via export to the Golgi complex, whereas malfolded and orphan protein subunits are retained within the ER lumen and eventually retranslocated to the cytoplasm and degraded by the ubiquitin/proteasome systems [7–11]. Various studies have also implicated the Sec61 complex as involved in retrotranslocation processes, i.e., the export of molecules from the ER [12,13]. For protein retrotranslocation, cytoplasmic factors are important and facilitate delivery of exported proteins to the proteasome [10].

Glycopeptide export is a model system for the study of retrograde ER transport in vitro [14–17]. Glycopeptide export can be monitored in a cell-free system by introducing into the ER an iodinated tripeptide (Ac–NYT–NH2) containing a canonical *N*-glycosylation sequon [14]. The addition of ATP and GTP is essential to achieve export, but there is no requirement for cytosol [15]. This pathway is biochemically closely related to protein export, suggesting that the in vitro system could allow identification of factors common to both peptide and protein mechanisms [16–18]. In an effort to extend characterisation of the in vitro system to identification of lumenal factors, we developed a novel reconstitution method which facilitates depletion of lumenal contents from purified microsomes by a mixed detergent procedure. The resulting microsomes retain glycosylation and export activity. Our data are consistent with a minimal role in glycopeptide export for ER lumenal components. In addition, we also demonstrate that controlled proteolysis can destroy export activity without rupturing the microsomes or depleting *N*-glycosylation, indicating that cytosolically exposed peptidic determinants are important for glycopeptide retrotranslocation.

2. Experimental procedures

2.1. Materials

Ac–NYT–NH2 was synthesised by Albachem (UK). ATP, GTP, GDP-mannose, Con A-Sepharose were from Sigma (UK). ^{125}I (100 mCi/ml) was from Amersham Pharmacia (UK). Creatine kinase and creatine phosphate were from Roche Diagnostics. Other chemicals were obtained from commercial sources and were of analytical grade or higher.

2.2. Preparation of rat liver microsomes

Rat livers were removed from adult rats and homogenised using a precooled dounce homogeniser in B88 buffer (20 mM HEPES–KOH, pH 7.4, 150 mM KOAc, 250 mM sorbitol, and 5 mM $Mg[OAc]_2$) containing 1 mM phenylmethylsulphonyl fluoride (PMSF) and 1 mM DTT at 4 °C. All subsequent manipulations were carried out at 4 °C unless indicated otherwise. The homogenate was centrifuged at 8000×*g* for 10 min to sediment large cellular debris, the supernatant was removed and centrifuged for a further 15 min at 8000×*g*. The supernatant was again centrifuged at 100 000×*g* for 40 min. The final microsomal pellet was resuspended in B88 buffer containing 1 mM DTT and snap-frozen in 100 μl (A_{280} ~200) aliquots in liquid nitrogen and stored at −80 °C.

2.3. Glycopeptide export assay from rat liver microsomes and peptide iodination

This was performed exactly as described [16].

2.4. Mild detergent permeabilisation of rat liver microsomes

A 100 μl aliquot of microsomes was washed in B88 buffer containing 0.5M NaCl and then resuspended in 300 μl B88 buffer. Fifty microliter samples were removed and incubated with an equal volume of CHAPS (Pierce) or saponin (Fluka) or a combination in B88 buffer and left on ice for 20 min. One milliliter of B88 buffer was then added to each sample to dilute the detergent, and the membranes were recovered by centrifugation. The microsomes were resuspended in 100 μl B88 buffer and 25 μl removed for Western analysis. The remaining 75 μl was tested for glycopeptide export activity.

2.5. Protease treatment of rat liver microsomes

A fresh aliquot of rat liver microsomes was thawed on ice and resuspended in B88 buffer containing 0.5M NaCl and rotated at 4 °C for 15 min. The membranes were collected by centrifugation at 20 000×*g* for 5 min in a microcentrifuge. The microsomes were resuspended in 1 ml B88 buffer and collected again by centrifugation and then resuspended in 500 μl B88. Proteases (trypsin, chymotrypsin, proteinase K, or

endoproteinase Glu-C, V8) were added from stock solutions to 100 μl aliquots of the resuspended microsomes to the desired final concentration. Proteolysis reactions were performed on ice for 10 min for trypsin, chymotrypsin, and proteinase K or at 37 °C for V8. Protease inhibitors were added to quench the reactions, and the mixtures were rapidly diluted in 1.5 ml of B88 buffer and the membranes collected by centrifugation for 5 min at 20000×*g*. The microsomes were resuspended in 100 μl B88, the 25 μl taken for Western blot analysis, and the remaining 75 μl used for assay of export and glycosylation.

2.6. Immunochemistry

Microsomes were fractionated by electrophoresis through 12% polyacrylamide reducing SDS gels [20]. Proteins were transferred onto nitrocellulose membranes in a wet electrotransfer cell (Amersham Pharmacia) overnight [21]. The membrane was stained with Ponceau S solution (Sigma) to confirm that transfer had occurred and was then incubated in blocking buffer [5% milk, 100 mM Tris–HCl pH 7.5, 0.9% NaCl (TTBS)] for 1 h. The membranes were probed with antibodies against ribophorin I (RI), PDI, Sec61α, BiP (Bioquote), SRPα, Sec62, or ribosomal protein S6 (Santa Cruz) followed by HRP-conjugated secondary antibody (Sigma) and visualised by enhanced chemiluminescence. Chemiluminescence fluorograms were quantitated by scanning of the X-ray films following exposure using a Heidelberg Linoscan 1400 32-bit scanner, followed by densitometric measurement using ImageJ on a Macintosh computer.

2.7. Quantitation of N-glycosylation activity

Residual glycosylation of protease-treated detergent reconstituted microsomes was evaluated by determining the total glycopeptide recovered by Con A capture of the treated sample relative to that recovered from an equivalent amount of untreated microsomes after incubation under identical conditions. The untreated sample was designated as 100%.

3. Results

3.1. Reconstitution of glycopeptide export from detergent semipermeabilised microsomes

Reconstitution of detergent solubilised microsomes capable of translocating proteins into the ER lumen has been achieved by solubilisation in cholate and other detergents followed by dialysis [23,24]. However, this treatment results in complete loss of *N*-glycosylation activity. *N*-glycosylation activity is both fundamental to analysis of glycopeptides but more importantly provides a potent indication that the microsomes retain a significant fraction of their native functionality, and hence are, by this important parameter at least, intact.

We initially evaluated treatment at high pH for opening the microsome membrane followed by resealing at neutral pH [25]. Although this approach gave some retention of

N-glycosylation and export competence, the level of activity was low (~5% starting activity, data not shown). Therefore, we sought to devise an alternate procedure for permeabilising and resealing the microsome membrane, specifically, using detergent [19]. Saponin is widely used for semipermeabilisation of cell membranes and has been exploited successfully for reconstitution of translocation and glycosylation of human interferon-γ into canine microsomes in vitro [26].

We titrated saponin and assayed the effect on both *N*-glycosylation and glycopeptide export activities in our microsomes. We performed the analysis with 30-min incubation, previously shown by us to be at the steady state level, i.e., after *N*-glycosylation and export is >85% completed, and hence a measure of overall efficiency and not rate [16]. *N*-glycosylation was very sensitive to detergent treatment, with a rapid loss of activity such that less than 15% activity was retained with saponin >0.1%. Glycopeptide export was rather less sensitive to detergent (Fig. 1). We routinely included a control reaction which lacks GTP, ATP, or both nucleotidetriphosphates; under these conditions, export is typically <10% of the full reaction during the 30-min incubation period. This background value is subtracted from all complete export assay data.

By Western analysis, we found that the two major soluble lumenal marker proteins of the ER, PDI, and BiP were removed by extraction with the higher concentrations of saponin, with release of the former somewhat more efficient than BiP (Fig. 1; <5% residual PDI, ~10% residual BiP as judged by densitometry). Ribophorin I was not extracted, indicating that the membranes had not been solubilised under these conditions. These data indicate that although both PDI and BiP are soluble ER lumenal proteins, they are differentially released from the microsomes, probably due to tighter association of BiP with other protein components in the ER (see below). At the upper end of the saponin concentrations tested, *N*-glycosylation efficiency was very low suggesting extensive damage to the oligosaccharyltransferase and/or other components (Fig. 1 and data not shown).

Therefore, we tested additional detergent systems. Of these, CHAPS used in combination with saponin gave selective release of lumenal components without extensive membrane solubilisation as monitored with ribophorin I (Fig. 2). At 0.075% (w/v) saponin alone, microsomes retained ~15% *N*-glycosylation activity, and again PDI and BiP were released, with PDI release more efficient. However, when CHAPS was also added, *N*-glycosylation activity in the microsomes was restored to ~70% together with increased retention of lumenal PDI and BiP, suggesting that the membranes were not solubilised to the same degree as with saponin alone. Presumably, as ribophorin I was not solubilised under any conditions, release of lumenal components was due to the introduction of pores into the membrane rather than disruption of the lipid bilayer. At higher concentrations of CHAPS, we observed removal of PDI to undetectable levels. *N*-glycosylation was retained at about 25% of control levels whilst export at 20% (Fig. 2). Loss of export activity did not correlate with the level of BiP retained by the microsomes as loss of substantial levels of BiP (compare CHAPS at 0.1% and 0.125%) had only a small effect on export. Hence either a small pool of BiP and/or PDI is sufficient for retention of export activity, or these two proteins play only a minimal role in the retrotranslocation of glycopeptide. However, it is clear that the vast majority of BiP and PDI, and hence most likely other microsomal lumenal proteins, may be removed without complete loss of export activity.

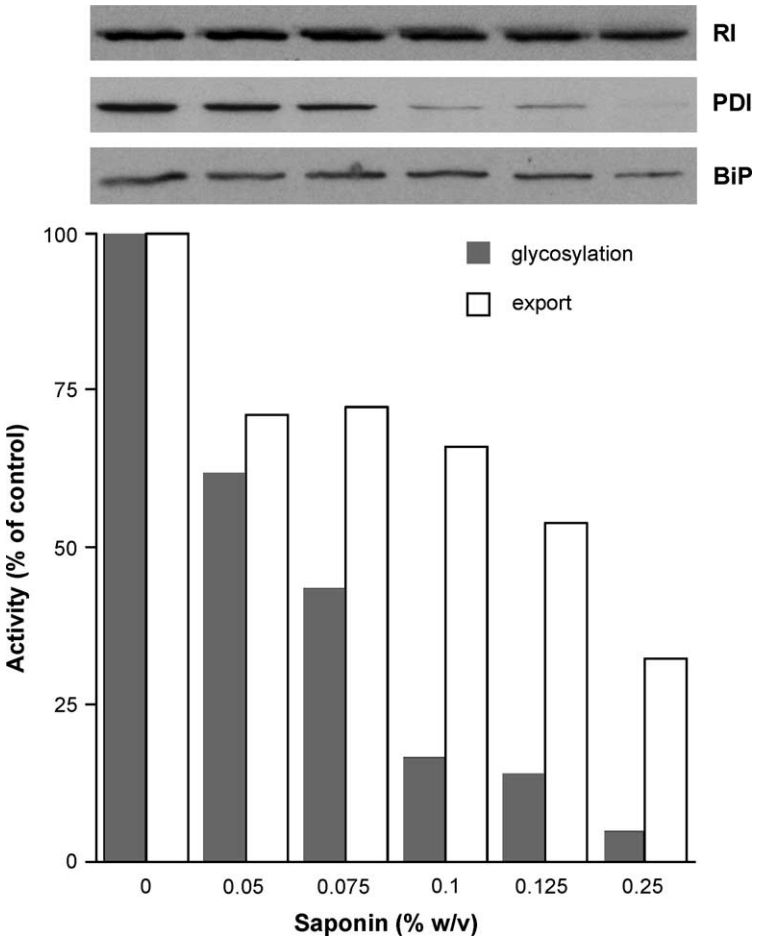

Fig. 1. Extraction of ER lumenal proteins with saponin. Salt-washed rat liver microsomes were incubated for 10 min on ice with increasing amounts of Saponin [0–0.25% (w/v)]. The detergent was diluted with B88 buffer and the microsomes pelleted by centrifugation. Residual glycosylation (grey bars) and glycopeptide export (white bars) activities were determined. Inset (top): equivalent amounts from each membrane sample were fractionated by SDS-PAGE, transferred onto nitrocellulose membranes, and probed with antibodies against ribophorin (RI), PDI, and BiP. PDI is more readily released than BiP. RI on the other hand was not affected indicating that only soluble proteins are released under these mild detergent treatments. A representative experiment of at least two is shown. It is important to note that in these assays the level of export is normalized for the total amount of glycosylation obtained with the treated membranes in that sample and is not therefore influenced by the decrease in *N*-glycosylation activity per se, i.e., export represents the proportion of glycopeptide exported as a fraction of the total glycosylated (see methods for details and Ref. [16]).

To investigate the mechanism underlying the lower efficiency of BiP extraction, we considered that BiP is generally active and binds substrate when in the ADP-bound form [27] and is expected to be more resistant to extraction with detergents. Adding ATP was expected to increase levels of ATP-BiP and hence the fraction of extractable

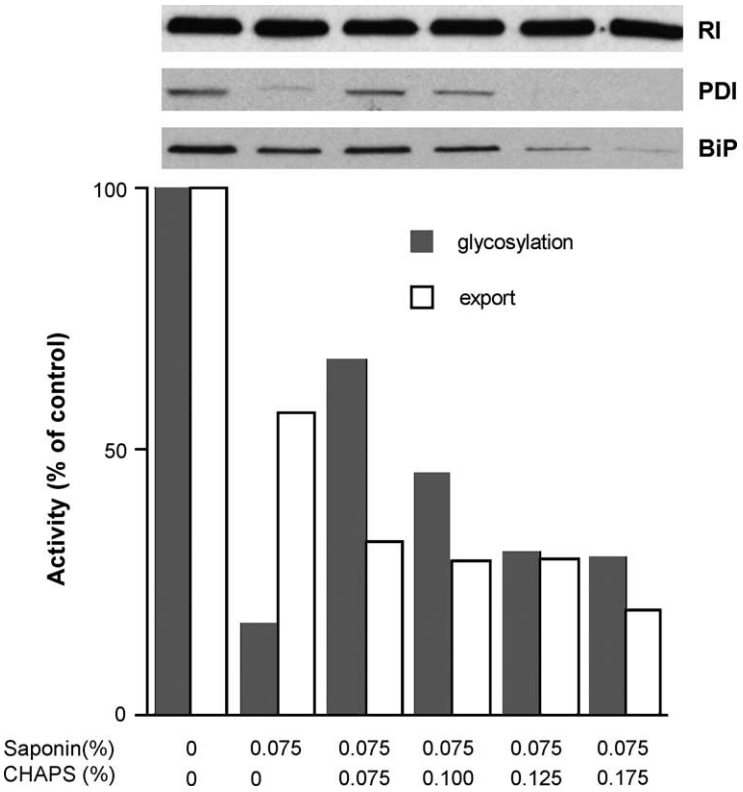

Fig. 2. A mixed detergent system improves retention of glycosylation and export activity. Glycopeptide export (white bars) and glycosylation (grey bars) activities are more stable when a combination of saponin and CHAPS were used for partial solubilisation of rat liver membranes. The saponin concentration was fixed at 0.075% (w/v), whereas CHAPS was varied [0–0.175% (w/v)]. Reconstitution involved resuspending the salt-washed microsomes in B88 buffer containing the detergents at the final concentrations designated in the graph, then removal of the detergent by simple dilution and pelleting the microsomes by centrifugation. The glycopeptide export activity is expressed as a percentage relative to untreated microsomes and corrected for the background release as a result of incubating the glycopeptide loaded microsomes with ATP only. Inset (top): Western blot analysis for RI, PDI, and BiP of the reconstituted microsomes in A. A representative experiment of two is shown.

BiP. When we added 1 mM ATP significantly less BiP was retained in the microsomes, although ribophorin levels were unaltered (compare lanes 2–4 with lanes 5–7 in Fig. 3). These microsomes also had decreased glycopeptide export activity, but this did not correlate with levels of BiP retained by the microsomes (Fig. 3). For example, 50% of export was lost by detergent addition (Fig. 3, lane 1 versus 2) with complete retention of BiP, whilst further loss of BiP by increasing detergent either with or without ATP (Fig. 3, lanes 2–4, 5–7) had no additional impact on export. Taken together, these data suggest that bulk BiP is not required for glycopeptide export, but do not rule out a role for a residual, extraction resistant minor pool. An alternate possibility is that the very small levels of radiolabelled export substrate used

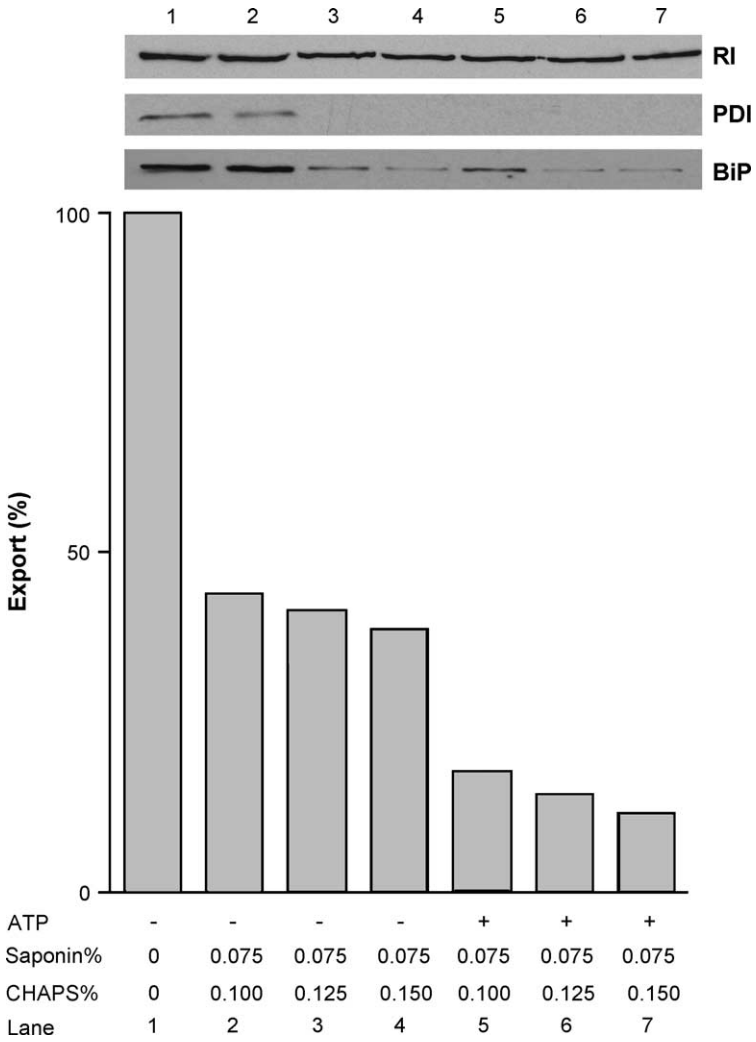

Fig. 3. Effect of ATP on BiP release and residual glycopeptide export activity. ATP was added at 1 mM to the partial solubilisation medium, which consisted of salt-washed microsomes, B88 buffer, 0.075% (w/v) saponin, and varying amounts of CHAPS [0.100–0.150% (w/v)]. Main panel: residual glycopeptide export activity of the reconstituted membranes; lane 1: microsomes treated with B88 alone; lanes 2–5: microsomes treated with 0.075% saponin and increasing amounts of CHAPS without ATP; and lanes 5–7: are as lanes 2–5 except that 1 mM ATP was present in the partial solubilisation step. Export activity was normalised to untreated membranes (control, lane 1) and corrected for the background level obtained when ATP alone was present during the export step. Inset (top): Western blot analysis of the reconstituted membranes for RI, PDI, and BiP. A representative experiment of three is shown.

here allows detection of export in the presence of vanishing (undetectable) levels of luminal chaperones—due to the manner in which the export assay is designed this possibility could not be explored further with the current system.

3.2. Proteolysis inhibition of glycopeptide export

Proteolysis has been utilised to probe the structure and function of microsomes in considerable detail [22]. We previously reported that glycopeptide export activity was abolished by mild trypsin treatment of rat liver microsomes, indicating the presence of protease-sensitive components on the external face of the microsomes [16]. We extended our study further to include additional proteases and also to monitor the effects of protease treatment on lumenal and membrane ER proteins. Pretreatment of rat liver microsomes with trypsin, chymotrypsin (data not shown), proteinase K, or V8 (data not shown) resulted in a dose-dependent loss of glycopeptide export activity but not import, *N*-glycosylation, or retention of the resultant glycopeptide in the treated microsomes in the absence of exogenous ATP and GTP (Fig. 4). Retention and stability of glycosylated peptide plus the Western blot analysis indicating full retention of PDI and ribophorin I (Fig. 4) by the microsomes are good evidence that the

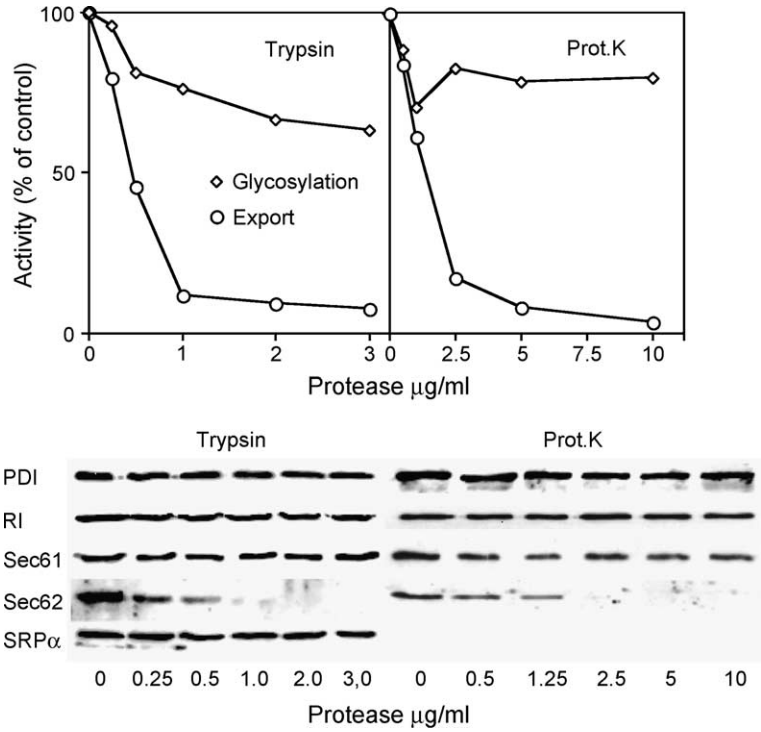

Fig. 4. Controlled proteolysis of microsomes ablates glycopeptide export. Salt-washed microsomes were resuspended in B88 buffer and proteases added. Membranes were exposed to trypsin or proteinase K (Prot. K) for 10 min on ice. Glycopeptide export (circles) and glycosylation (diamonds) of the treated microsomes were then evaluated relative to the untreated (control) samples. Western blot analysis of a panel of ER-associated proteins from trypsin and proteinase K-treated microsomes is also shown. Protein disulphide isomerase (PDI) and ribophorin I (RI) represent ER lumenal soluble and integral membrane proteins, respectively, whereas Sec61, Sec62, and SRPα represent translocon components and the signal recognition particle α-chain.

integrity of the membranes is unaffected by the protease treatment, and also that import of the peptide and export of the glycopeptide have distinct requirements. Proteinase K (2.5 μg/ml) and trypsin (1 μg/ml) treatments resulted in loss of export activity, whilst over 80% of glycosylation and retention of glycopeptide was maintained. Similar data were obtained for V8 and chymotrypsin (data not shown). Hence, controlled proteolysis discriminates between components involved in import, export, and glycosylation. Significantly, Sec61α was unaffected by either trypsin or proteinase K, even at concentrations fully inhibiting export, and signal recognition particle receptor (SRPR) α-subunit was unaffected by trypsin. By contrast, both trypsin and proteinase K degraded Sec62. Most notably, the level of Sec62 correlated well with residual microsomal export activity (Fig. 4). These data indicate that protein components involved in glycopeptide export are sensitive to external proteases and hence have cytosolically exposed determinants.

4. Discussion

Export of proteins and peptides from the ER constitutes an important part of the quality control mechanism of the mammalian secretory system, plus it is an important component of immunological surveillence systems for viral antigens [4]. Previously, we demonstrated that, for glycotripeptides, there is no requirement for cytosolic protein factors, and that the only requirement for retrotranslocation across the ER membrane appears to be GTP and ATP [14,15]. The site(s) of action of these two nucleotide triphosphates remain unknown, although several GTPases and ATPases are part of the ER translocation system. Additional work has also demonstrated that the translocon, through which glycopeptides are transported, is the Sec61 complex [1,12,13]. We demonstrate here that highly specific proteolytic treatment of intact microsomes ablates export competence without reducing *N*-glycosylation or releasing lumenal proteins. Interestingly, loss of export competence correlates with degradation of Sec62, consistent with a requirement for an intact Sec61 complex for retrotranslocation. Overall, these data provide additional evidence for the role of membrane-embedded proteins as having a role in glycopeptide retrotranslocation, and in particular, that some of these factors must maintain cytosolically exposed determinants due to their accessibility to protease.

Despite many advances in mapping ER retrotranslocation pathways, the role of lumenal components has been less well characterised, in part due to the difficulty of accessing this compartment for biochemical purposes without compromising microsomal function. Most significantly, we report a mixed detergent system that retains a substantial level of export and glycosylation activity. It is of interest that it was necessary to use two distinct detergents to obtain sufficient solubilisation with retention of function; we assume that the addition of CHAPS to the system provides a more native environment for protection of ER membrane components, compared with saponin alone. This technique facilitated removal of >95% of lumenal protein, as accessed by using two high-abundance ER lumen proteins, BiP and PDI. Clearly, the vast majority of these proteins can be removed with retention of activity, although it

must be bourne in mind that some luminal proteins may not have been extracted, and even the two proteins studied, BiP and PDI, demonstrated differential extractability. Whilst the data do not formally rule out a role for BiP and PDI, they are suggestive of a lack of direct involvement in tripeptide export. Due to the small size of the export substrate used here, it is unlikely that a chaperone function of BiP is required, although in the case of larger peptides, this may not be the case, as BiP is clearly required for protein retrotranslocation. Furthermore, the minute levels of radiotracer used for our assays may also facilitate detection of export, whereas transport of substrates at more physiological concentrations may be compromised. However, the reconstitution method described here does allow removal of lumenal components with retention of *N*-glycosylation and hence provides a new approach for biochemical analysis of microsome function.

Acknowledgments

This work was supported by the BBSRC (Project grant 28/C09893 to MCF). We are indebted to Chris Nicchitta, Martin Jung, Enno Hartmann, Peter Walter, and Colin Stirling for generous provision of antibodies. MCF wishes to dedicate this paper to the memory of Dr. Gary Warren.

References

[1] Johnson AE, van Waes MA. The translocon: a dynamic gateway at the ER membrane. Annu Rev Cell Dev Biol 1999;15:799–842.
[2] Heinrich SU, Mothes W, Brunner J, Rapoport TA. The Sec61p complex mediates the integration of a membrane protein by allowing lipid partitioning of the transmembrane domain. Cell 2000;102: 233–44.
[3] Crowley KS, Liao S, Worrell VE, Reinhart GD, Johnson AE. Secretory proteins move through the endoplasmic reticulum membrane via an aqueous, gated pore. Cell 1994;78:461–71.
[4] Hurtley SM, Helenius A. Protein oligomerisation in the endoplasmic reticulum. Annu Rev Cell Biol 1989;5:277–307.
[5] Gaut JR, Hendershot LM. The modification and assembly of proteins in the endoplasmic reticulum. Curr Opin Cell Biol 1993;5:589–95.
[6] Hammond C, Helenius A. Quality control in the secretory pathway. Curr Biol 1995;7:523–9.
[7] Kopito RR. ER quality control: the cytoplasmic connection. Cell 1997;88:427–30.
[8] Ellgaard L, Molinari M, Helenius A. Setting the standards: quality control in the secretory pathway. Science 1999;286:1882–8.
[9] Bonifacino SJ, Weissman AM. Ubiquitin and the control of protein fate in the secretory and endocytic pathways. Annu Rev Cell Dev Biol 1998;14:19–57.
[10] Brodsky JL, McCracken AA. ER quality control and proteasome-mediated protein degradation. Semin Cell Dev Biol 1999;10:507–13.
[11] Lord JM, Davey J, Freigero L, Roberts LM. Endoplasmic reticulum-associated protein degradation. Semin Cell Dev Biol 2000;11:159–64.
[12] Wiertz EJHJ, Tortorella D, Bogyo M, Yu J, Mothes W, Jones TR, Rapoport TA, Ploegh HL. Sec61 mediates transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. Nature 1996;384:432–8.

[13] Romisch K. Surfing the Sec61 channel: bidirectional protein translocation across the ER membrane. J Cell Sci 1999;112:4185–91.
[14] Romisch K, Schekman R. Distinct processes mediate glycoprotein and glycopeptide export from the endoplasmic reticulum in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 1992;89:7227–31.
[15] Romisch K, Ali BRS. Similar processes mediate glycopeptide export from the endoplasmic reticulum in mammalian cells and *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 1997;94:6730–4.
[16] Ali BRS, Field MC. Glycopeptide export from mammalian microsomes is independent of calcium and is distinct from oligosaccharide export. Glycobiology 2000;10:383–91.
[17] Ali BRS, Tjernberg A, Chait B, Field MC. A microsomal GTPase is required for glycopeptide export from the mammalian endoplasmic reticulum. J Biol Chem 2000;275:33222–30.
[18] Gillece P, Pilon M, Romisch K. The protein translocation channel mediates glycopeptide export across the endoplasmic reticulum membrane. Proc Natl Acad Sci U S A 2000;97:4609–14.
[19] Paver JL, Hawkins HC, Freedman RB. Preparation and characterization of dog pancreas microsomal membranes specifically depleted of protein disulphide-isomerase. Biochem J 1989;257:657–63.
[20] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
[21] Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 1979;76:4350–4.
[22] Song W, Raden D, Mandon EC, Gilmore R. Role of Sec61 in the regulated transfer of the ribosome-nascent chain complex from the signal recognition particle to the translocation channel. Cell 2000; 100:333–43.
[23] Nicchitta CV, Migliaccio G, Blobel G. Biochemical fractionation and assembly of the membrane components that mediate nascent chain targeting and translocation. Cell 1991;65:587–98.
[24] Nicchitta CV, Blobel G. Assembly of translocation-competent proteoliposomes from detergent-solubilized rough microsomes. Cell 1990;60:259–69.
[25] Bulleid NJ, Freedman RB. Defective co-translational formation of disluphide-isomerase-deficient microsomes. Nature 1988;335:649–51.
[26] Bulleid NJ, Freedman RB. Cotranslational glycosylation of proteins in systems depleted of proteins disulphide isomerase. EMBO J 1990;9:3527–32.
[27] Hendershot L, Wei J, Gaut J, Melnick J, Aviel S, Argon Y. Inhibition of immunoglobulin folding and secretion by dominant negative BiP ATPase mutants. Proc Natl Acad Sci U S A 1996;93:5269–74.