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

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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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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.
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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.

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