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Reconstitution of Protein Translocation Activity from Partially Solubilized Microsomal Vesicles*

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We have used a reconstitution assay to demonstrate that protein translocation activity can be recovered after microsomal vesicles derived from the rough endoplasmic reticulum have been partially solubilized with *n*-octyl-β-glucopyranoside. Two independent approaches were used to establish conditions for partially solubilizing microsomal membranes. When the lipid bilayer was disrupted by detergents to the extent that the integrity of the lipid bilayer had been perturbed, membranes were inactive for translocation. However, detergent-treated membranes could be reconstituted in good yield into a translocation competent form once the detergent was removed.

In higher eukaryotes, ribosomes synthesizing secretory and some integral membrane proteins are specifically targeted to the membrane of the rough endoplasmic reticulum (RER)[1] (1). These ribosomes become bound to the membrane, and the nascent protein chains they synthesize are translocated across the RER membrane (2). The events and components that facilitate ribosome targeting have been well characterized (3). However, very little is known about how nascent chains are translocated into the lumen of the RER and which membrane proteins facilitate this process.

The translocation of nascent chains is likely to involve the concerted action of a complex assembly of RER membrane proteins, termed translocon (3). Some of these proteins may play an active role in facilitating the movement of the nascent chain across the membrane by serving as a protein motor and/or a proteinaceous tunnel (2). Other proteins may help to target and anchor ribosomes to the membrane or may enzymatically modify the nascent chain but may not themselves contribute to its vectorial movement. To date, only two RER membrane proteins with known roles in this translocation process have been purified, the SRP receptor (4, 5) and signal peptidase (6). Other membrane proteins have recently been shown by photoaffinity labeling to be in close proximity to the nascent chain as it is translocated across the membrane (7, 8). Since these proteins are integral membrane proteins that are in intimate contact with the nascent chain as it spans the membrane, they are thought to play a more direct role in the translocation of the nascent chain.

Despite the progress that has been made in recent years in identifying membrane proteins that participate in translocation, further analysis of the role that these and other proteins play in this process remains a formidable task. Such analysis would be greatly facilitated if the translocation assembly could be reconstituted from detergent extracts of microsomal vesicles. As a first step toward developing a strategy for such a reconstitution, we wanted to determine whether translocation activity can be recovered after the integrity of the RER membrane has been highly disrupted by detergent. In the current work we have treated microsomal vesicles with sufficient detergent to partially solubilize the membranes and have developed a method for recovering sealed vesicles from the detergent-disrupted microsomes. We have shown that although partially solubilized microsomes are incompetent for translocation, translocation competence can be restored to the membranes once the detergent is removed.

EXPERIMENTAL PROCEDURES

Materials—[^{35}S]Methionine (800 Ci/mmol) was purchased from Amersham Corp.; Nikkol (octaethyleneglycol mono-*n*-dodecyl ether) was from Nikko Chemicals Co., Ltd., Tokyo, Japan; hydroxylapatite (Bio-Gel HTP) was from Bio-Rad; ConA-Sepharose was from Pharmacia, Uppsala, Sweden; and methyl α-D-mannopyranoside, *n*-octyl β-glucopyranoside (OG), and L-α-phosphatidylcholine (PtdCho) were from Sigma.

Preparation of Salt-extracted and EDTA-stripped Microsomal Membranes—Canine rough microsomes that were EDTA-stripped and/or salt-extracted were prepared as described previously (9).

Purification of Signal Peptidase—Salt-extracted rough microsomes were incubated at a final concentration of 0.5 eq/μl in a buffer containing 150 mM KOAc, 50 mM triethanolamine-HOAc, pH 7.5 (TEA), 1 mM dithiothreitol (DTT), 1 mM Nikkol on ice for 30 min. One equivalent is defined as the material derived from 1 μl of rough microsomal membranes that are at a concentration of 50 A_{280} units/ml (9). The detergent extract (16 ml) was underlayered with 8 ml of cushion (50 mM TEA, 500 mM sucrose, 150 mM KOAc, 1 mM DTT) and centrifuged for 30 min at 45,000 rpm (184,000 × g_{av}) in a Beckman Ti-50.2 rotor. The pellet fraction was resuspended in 8 ml of a buffer containing 526 mM KOAc, 53 mM TEA, 1 mM DTT, 21 mM sodium phosphate, pH 6.8, 10% glycerol with a Dounce homogenizer, and 400 μl of 20% Nikkol (1% final) was slowly added to the suspension under constant agitation. After a 30-min incubation on ice the detergent suspension was centrifuged at 40,000 rpm (100,000 × g_{av}) for 2 h in a Ti-50.2 rotor. The supernatant was collected and applied to a hydroxylapatite column (1 ml of resin for each 10 ml of supernatant) equilibrated with a buffer containing 50 mM TEA, 500 mM KOAc, 1 mM DTT, 20 mM sodium phosphate, pH 6.8, 0.1% Nikkol, 10% glycerol.

The flow-through fraction from the hydroxylapatite column was loaded (8 ml/h) onto a ConA-Sepharose column (1 ml of resin for each 8 ml of sample) equilibrated with the same buffer. The column was washed with 2 column volumes of a buffer containing 50 mM TEA, 100 mM KOAc, 1 mM DTT, 0.4% Nikkol and eluted (2 ml/h) with 2 column volumes of a buffer containing 50 mM TEA, 100 mM KOAc, 1 mM DTT, 250 mM sucrose, 750 mM methyl α-D-mannopyr-

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[1] The abbreviations used are: RER, rough endoplasmic reticulum; OG, *n*-octyl-β-glucopyranoside; TEA, triethanolamine; DTT, dithiothreitol; ConA, concanavalin A; SRP, signal recognition particle; PtdCho, phosphatidylcholine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ER, endoplasmic reticulum.

anoside, 0.4 mg/ml PtdCho. The eluent was brought to 15 mM sodium phosphate, diluted 3-fold with a buffer containing 50 mM TEA, 100 mM KOAc, 1 mM DTT, 100 mM sucrose, 0.4% Nikkol, 0.4 mg/ml PtdCho, adjusted to pH 6.8, and loaded onto a hydroxylapatite column (150 μl of resin for each 10 ml of sample) equilibrated with the same buffer. Only about half of the signal peptidase bound to this column. The flow-through fraction was reloaded onto a second hydroxylapatite column (150 μl of resin for each 10 ml of sample) equilibrated with the same buffer. The second column was eluted with 1 column volume of a buffer containing 50 mM TEA, 500 mM KOAc, 250 mM sucrose, 10 mM sodium phosphate, pH 6.8, 0.4% Nikkol, 0.4 mg/ml PtdCho and contained essentially homogeneous signal peptidase. From 1000 eq of rough microsomes, 40 ng of homogeneous signal peptidase was obtained. This is comparable with the yield from the previously reported purification (6). Each microgram of the purified protein complex contained approximately 25 units of activity (6).

ConA Blots—In vitro ^{14}C labeling of ConA was done by reductive methylation as reported (10). After transfer of the protein to nitrocellulose (10), the blots were blocked for 30 min with ConA buffer (50 mM Tris-HCl, pH 7.5, 140 mM NaCl, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM $CaCl_2$, 0.02% NaN_3) containing 1% (w/v) bovine hemoglobin. The blots were then incubated overnight with the same buffer containing 15 μg of [^{14}C]ConA (2000 cpm/μg) per lane of proteins on the nitrocellulose filter. After the incubation, blots were washed three times, 10 min each wash, with 140 mM NaCl, dried under a lamp, and exposed directly to Kodak X-Omatic AR film.

Turbidity Measurements—Salt-washed and EDTA-stripped microsomes were incubated at a final concentration of 1 eq/ml in a buffer containing 125 mM sucrose, 50 mM TEA, 150 mM KOAc, 1 mM $Mg(OAc)_2$, 1 mM DTT containing the appropriate concentration of OG. After a 30-min incubation on ice, 50 μl of sample was diluted into 550 μl of the same buffer without detergent, and the absorbance was measured at 500 nm with a spectrophotometer.

Detergent Treatment and Reconstitution of Stripped Microsomal Membranes—Salt-washed and EDTA-stripped microsomes were incubated at a final concentration of 1 eq/μl in 125 mM sucrose, 50 mM TEA, 150 mM KOAc, 1 mM $Mg(OAc)_2$, 1 mM DTT containing the appropriate concentration of OG. After a 30-min incubation on ice the extracts were diluted 10-fold with cold buffer. The diluted samples were centrifuged for 5 min at 80,000 rpm (228,000 × g_{av}) in a Beckman TL-100.2 rotor at 4 °C. Supernatant fractions were saved for analysis by SDS-PAGE. Pellet fractions were resuspended in two times the original volume of cold 50 mM TEA, 250 mM sucrose, 1 mM DTT and were again centrifuged at 80,000 rpm for 5 min. The pellet fractions were resuspended to a final concentration of 2 eq/μl in 50 mM TEA, 250 mM sucrose, 1 mM DTT. Control membranes containing no OG underwent the same treatment as detergent-treated membranes. For the assays described here we found some slight variability between membrane preparations (not shown), and the exact OG concentrations required to get the desired degree of solubilization or disruption were determined empirically for each batch of microsomes.

The samples shown in Fig. 2 were detergent-treated as described above, and, after a 30-min incubation on ice, 75 μl of extract was centrifuged at 25 p.s.i. for 3 min in an A-110 rotor in a Beckman airfuge.

Translocation Assays—Wheat germ translation extracts and SRP were prepared as described previously (11, 12). Translations were programmed with preprolactin mRNA as described (13), except that RNA transcripts obtained from 2.5 ng of plasmid (contained in 1 μl) were translated in each 10 μl of reaction containing 25 μCi of [^{35}S] methionine. In the reactions shown in Fig. 5, 4 eq of membranes were included per 20 μl of reaction and, where indicated, 5 mM OG was included in the reactions. The ionic conditions were kept constant in all the reactions. Aliquots of each reaction were used for protease protection assays or were prepared for SDS-PAGE as described (14). Protease protection assays were done as reported previously (15).

RESULTS

Before attempting to reconstitute protein translocation activity from detergent extracts of microsomal membranes, we established conditions whereby all the microsomes in a suspension would be disrupted by detergent. To analyze the extent of microsome disruption, a turbidity assay was used to follow the solubilization of the lipid bilayer by detergent, and a blotting assay was used to measure the concentration of detergent required to release the lumenal contents from the microsomes or to solubilize an ER membrane protein known to be involved in translocation.

Measuring the turbidity, or optical density, of a membrane-detergent suspension is a commonly used method for determining the extent of solubilization of membrane vesicles (16). A three-stage model has been proposed to describe the solubilization of lamellar structures into mixed micelles (17), and these stages can be monitored by measuring the turbidity of treated membranes (18). Thus, by measuring turbidity as optical density at 500 nm, we were able to plot the stages of solubilization of microsomal vesicles as a function of detergent concentration (Fig. 1). The rise in turbidity that occurs between 0 and 13 mM OG in Fig. 1 represents the first stage of the solubilization process. Free detergent molecules partition between the aqueous medium and the lipid bilayer during this stage. The presence of detergent in the vesicles makes them larger, and this is thought to account for the increased turbidity (16, 17). Between 13 and 40 mM OG there is a rapid decrease in turbidity that represents stage II of the solubilization process. The bilayers are saturated with detergent, and lipid-detergent micelles begin to form as more lipids are extracted from the bilayer (17, 18). Note that the midpoint of this change occurs at around the critical micelle concentration of the detergent, *i.e.* the concentration of detergent above which micelles are formed (25 mM OG in aqueous solution). By stage III (around 50 mM OG in Fig. 1) the bilayers are completely solubilized; all the lipids are present in mixed lipid-detergent micelles, and the suspension is no longer turbid (16–18).

According to the data presented in Fig. 1, microsomal vesicles are at stage II of solubilization after treatment with 13–40 mM OG. Thus, their lipid bilayers are fully saturated with detergent and partially solubilized. Microsomes treated with OG concentrations in this range should still pellet after centrifugation carrying integral membrane proteins with them. Thus, as an independent measure of solubilization, we have followed the sedimentation behavior of a known membrane protein, the glycosylated subunit of signal peptidase.

Signal peptidase is an integral membrane protein complex of six polypeptides, which includes two glycoproteins (6). The glycoproteins migrate differently on SDS-PAGE but have identical amino acid sequences that contain a hydrophobic transmembrane region (19). These bands can be visualized by using [^{14}C]ConA to probe protein blots of either the purified signal peptidase complex (Fig. 2, *lane 4*) or the microsomal membranes (Fig. 2, *lane 3*) with [^{14}C]ConA (5). By following the signal peptidase polypeptides during our purification pro-

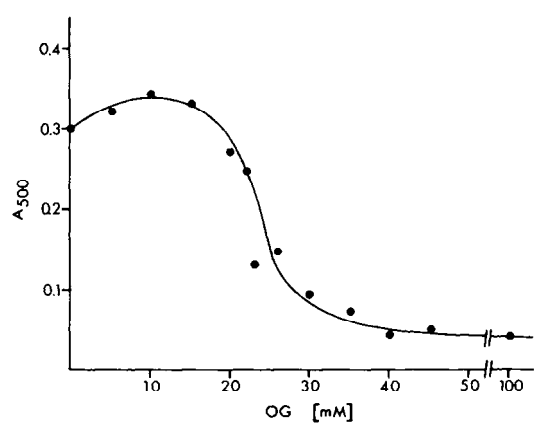

FIG. 1. **The effect of OG on the turbidity of a microsomal membrane suspension.** The turbidity (A_{500}) of salt-extracted and EDTA-stripped microsomes was measured after a 30-min incubation with various concentrations of OG (see "Experimental Procedures").

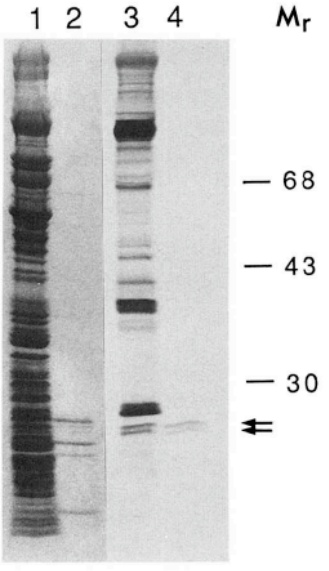

FIG. 2. **Purified signal peptidase compared with salt-extracted microsomal membranes.** Signal peptidase (*lanes 2* and *4*) was purified from salt-extracted microsomal membranes (*lanes 1* and *3*) as described (see "Experimental Procedures"). Twenty equivalents of microsomes (*lanes 1* and *3*) or 1 μg of purified signal peptidase (*lanes 2* and *4*) were resolved by SDS-PAGE on 10–15% gradient gels. After electrophoresis samples were visualized by Coomassie Blue staining (*lanes 1* and *2*) or were transferred to nitrocellulose, probed with [^{14}C]ConA, and visualized by exposure to X-Omat AR Kodak film (*lanes 3* and *4*) (see "Experimental Procedures"). The signal peptidase glycoproteins are indicated by a *double arrow*. Molecular weights (M_r) are indicated.

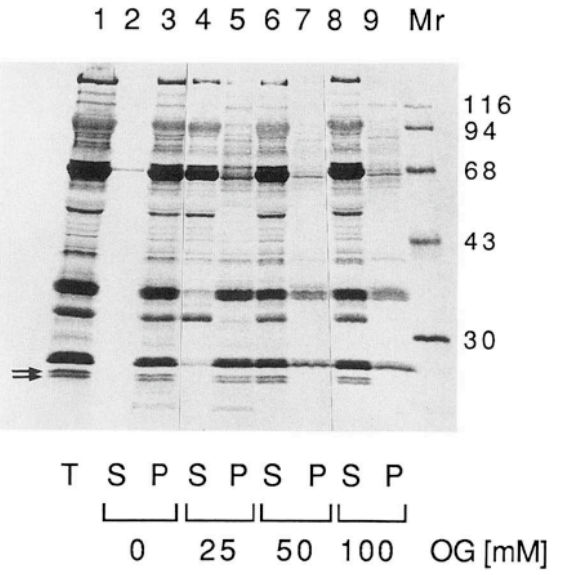

FIG. 3. **The release of lumenal proteins and the solubilization of membrane proteins from microsomal vesicles occur at different and discrete OG concentrations.** One hundred equivalents of salt-extracted microsomes were incubated with the indicated amount of OG and were centrifuged into supernatant (*S*) and pellet (*P*) fractions as described (see "Experimental Procedures"). Twenty equivalents of salt-extracted microsomes (*T*) (*lane 1*) or of each fraction (*lanes 2–9*) were resolved by electrophoresis on a 10–15% gradient gel in SDS. Proteins were blotted onto nitrocellulose, and the filter was probed with [^{14}C]ConA and exposed to X-Omat AR Kodak film. Note that the samples in *lanes 2* and *3* were subjected to the same treatment as the samples in *lanes 4–9* except that no OG was present in the suspension. Molecular weight standards (M_r) are indicated.

tocol, we determined that they are the only glycoproteins in microsomal membranes that migrate at 22 and 23 kDa on SDS-PAGE (data not shown). Thus, [^{14}C]ConA blots can be used to probe microsomal membrane fractions for the presence of the signal peptidase glycoproteins (indicated by a *double arrow*, compare Fig. 2, *lanes 3* and *4*). Since soluble glycoproteins can also be identified on [^{14}C]ConA blots (20), we also used this procedure to follow the behavior of the glycoproteins in the ER lumen after detergent treatment and fractionation of the microsomal membrane.

Microsomes incubated with increasing concentrations of OG were separated into supernatant and pellet fractions by centrifugation in an airfuge ("Experimental Procedures"). We then probed protein blots of each fraction with [^{14}C]ConA. We found that treatment of microsomes with 25 mM OG resulted in the release of lumenal glycoproteins (compare Fig. 3, *lane 4* to *lane 5*), without solubilizing integral membrane proteins such as signal peptidase (*double arrow*) and SRP receptor (localized by probing protein blots with monoclonal antibodies to both subunits (not shown)). In contrast, when membranes were treated with 50 mM OG, signal peptidase was recovered in the supernatant fraction (Fig. 3, *lane 6*), indicating that the lipid bilayer had been solubilized. We have shown by independent means that OG does not dissociate the signal peptidase complex (not shown). Thus, the presence of the signal peptidase glycoproteins in these fractions indicates that the whole complex has been solubilized.

Note that even after treatment with 50 mM OG some glycoproteins remained in the pellet fraction (Fig. 3, *lane 7*). By Western blotting we determined that both subunits of the SRP receptor were in the pellet fraction (data not shown). The amount of material in this fraction did not significantly decrease when a higher detergent concentration was used (Fig. 3, *lane 9*), and turbidity measurements confirmed that the vesicles were completely solubilized under these conditions (Fig. 1, see "Discussion"). Since under low ionic strength conditions the SRP receptor proteins pellet even in the absence of an intact lipid bilayer, they may comprise a network held together by protein-protein interactions.

Microsomes treated with OG concentrations near the critical micelle concentration of OG (25 mM) are saturated with detergent and disrupted, yet they still contain both signal peptidase and SRP receptor. We developed a simple procedure for recovering sealed membrane vesicles from this "extract." Microsomes were incubated with OG at or below 25 mM. The detergent concentration was then reduced 10-fold by diluting out the suspension with buffer containing no detergent, and the samples were separated into supernatant and pellet fractions by centrifugation. The pellet fractions were resuspended in detergent-free buffer and were recovered after a second centrifugation. The glycoproteins in each fraction were visualized by [^{14}C]ConA blotting (Fig. 4*A*). The amount of lumenal proteins released into the supernatant fraction increased with increasing detergent concentration (Fig. 4*A*, *lanes 2*, *4*, *6*, and *8*). The microsomes treated with 25 mM OG released most of their lumenal contents into the supernatant fraction (Fig. 4*A*, *lane 8*), indicating that the integrity of the vesicles was highly disrupted. By probing protein blots with antibodies against immunoglobulin heavy chain binding protein, a soluble protein which resides in ER lumen (21), we determined that the detergent-treated membranes contain less than 2% of the amount of immunoglobulin heavy chain binding protein normally present in rough microsomal vesicles (data not shown). However, as noted above, all the signal peptidase (indicated by a double arrow) remained in the pellet fraction (Fig. 4*A*, *lane 9*), indicating that the vesicles were not completely sol-

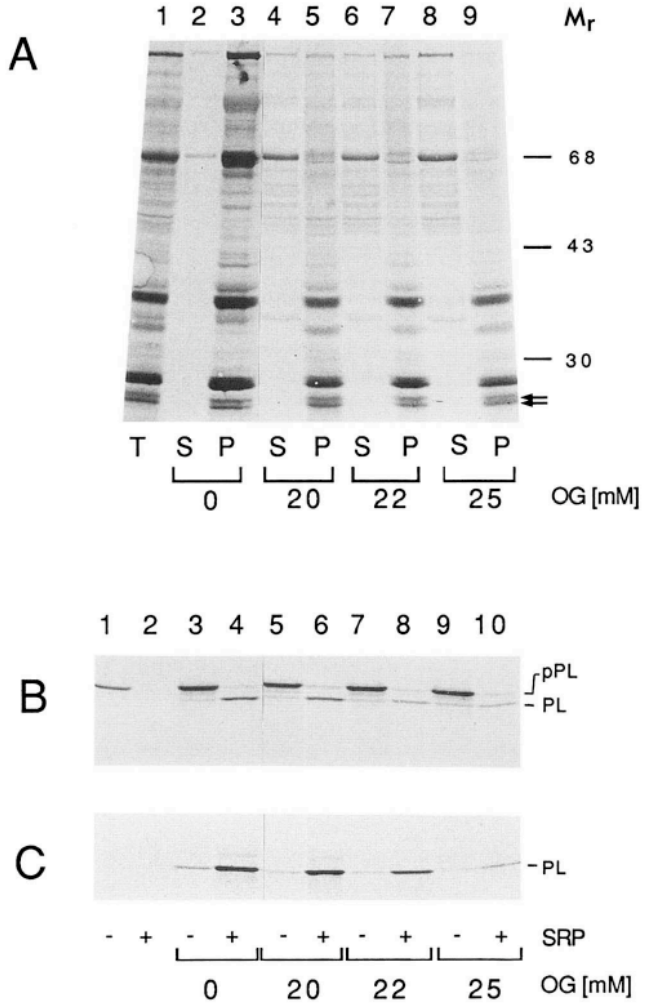

FIG. 4. **Translocation competent vesicles can be recovered from detergent-treated microsomes.** *A*, glycoprotein profile of fractions recovered after detergent treatment of microsomes. Supernatant (*S*) and pellet (*P*) fractions were processed as described (see "Experimental Procedures") and analyzed by [^{14}C]ConA blotting. Twenty equivalents of each fraction or total salt-extracted and EDTA-stripped microsomes (*T*) were loaded in each lane. The bands corresponding to signal peptidase are indicated with a *double arrow*. *B*, translocation reactions (see "Experimental Procedures") were carried out in the absence (*lanes 1* and *2*) or in the presence (*lanes 3–10*) of microsomes recovered after detergent treatment (see "Experimental Procedures"). *OG [mM]* indicates the concentration of OG which the membranes had been incubated with prior to their recovery after the detergent was diluted and removed. Membranes treated with no OG were processed in a similar manner to the detergent-treated membranes. SRP was included at 10 nM where indicated. The precursor protein, preprolactin (*pPL*), and processed prolactin (*PL*) are indicated. *C*, an aliquot of each reaction in *B* was treated with protease K before being prepared for SDS-PAGE.

ubilized. By electron microscopy we have shown that reconstituted vesicles are unilamellar and have a similar morphology to the starting membranes (not shown).

We tested the pellet fractions for activity in a co-translational translocation assay. Preprolactin was synthesized in a wheat germ translation extract (Fig. 4*B*, *lane 1*), and its synthesis was arrested by the addition of 10 nM SRP. SRP is required to target the nascent chain and ribosome to the RER membrane, but in the absence of added membranes it forms a ternary complex with the ribosome and nascent chain and arrests or slows further synthesis of the nascent chain (Fig. 4*B*, *lane 2*). When mock-treated microsomes were added to the translation extract, preprolactin was processed to prolactin in the presence of SRP (Fig. 4*B*, *lane 4*), revealing that the nascent protein chain had been transferred to the interior of the microsome and thereby became susceptible to cleavage by signal peptidase (1). Note that in the absence of added SRP some processing was detected (Fig. 4*B*, *lanes 3*, *5*, *7*, and *9*) due to a small amount of residual SRP present on the microsomes. Most importantly, when detergent-treated microsomes reconstituted by the procedure described above were included in the translation mixture, SRP-dependent processing of preprolactin to prolactin was also detected (Fig. 4*B*, *lanes 5–10*). The amount of processed prolactin decreased with increasing detergent concentration (Fig. 4*B*, *lanes 5–10*), so that for microsomes treated with 25 mM OG, the amount of protein translocation activity recovered appeared to be only slightly above background (Fig. 4*B*, *lanes 9* and *10*).

Signal peptidase normally cleaves proteins after they reach the interior of the microsome (2). However, detergent-extracted signal peptidase can cleave full length substrate proteins independently of ongoing translation or translocation (22). Although signal peptidase has not been solubilized in the assayed samples, the lumenal content proteins have been released, and hence, it is possible that the active site of signal peptidase has become exposed to proteins on the outside of the microsome. Thus, processing of preprolactin to prolactin may not be an adequate criteria for translocation. We therefore subjected aliquots of the translation extracts shown in Fig. 4*B* to digestion with protease K before preparing the samples for SDS-PAGE (Fig. 4*C*). Only prolactin which has been translocated into the lumen of a sealed vesicle should be protected from digestion by the protease (9). Translocation competent vesicles, as determined by protease protection of mature prolactin, were recovered from membranes treated with each concentration of detergent used (Fig. 4*C*, *lanes 3–10*). In this assay the microsomal membranes recovered after treatment with 25 mM OG are more clearly dependent on the presence of SRP for protein translocation (Fig. 4*C*, compare *lanes 9* and *10*). It seems that by subjecting the translation products to digestion with protease, translocated prolactin is distinguished from prolactin produced by a fraction of signal peptidase that loses its latency during the detergent treatment. At detergent concentrations above 25 mM OG, no translocation competent membranes were recovered (data not shown).

Taken together, the data in Figs. 1 and 3 strongly suggest that after treatment with 23–25 mM OG all the microsomal vesicles in the population are saturated with detergent and partially solubilized. We wanted to rule out the possibility that the recovered activity is derived from a small subset of vesicles in the extract that actually contains little or no detergent. If there were any vesicles in the extract that were not affected by the detergent then they should be active for translocation whether or not the detergent is subsequently removed. We therefore assayed aliquots of detergent-treated vesicles before the reconstitution procedure for translocation activity (Fig. 5).

Microsomes were treated with 23 mM OG, and aliquots were either assayed directly for translocation activity (Fig. 5, *lanes 3* and *4*) or were diluted and washed prior to being assayed (Fig. 5, *lanes 5* and *6*). Note that the detergent-depleted (*i.e.* reconstituted) membranes displayed enhanced processing of preprolactin to prolactin in the presence of SRP (Fig. 5*A*, compare *lanes 5* and *6*). The membrane fraction assayed before detergent removal displayed no enhanced processing in the presence of SRP (Fig. 5*A*, compare *lanes 3* and *4*). This indicates either that SRP targeting does not occur or that targeting occurs but does not result in a productive interaction

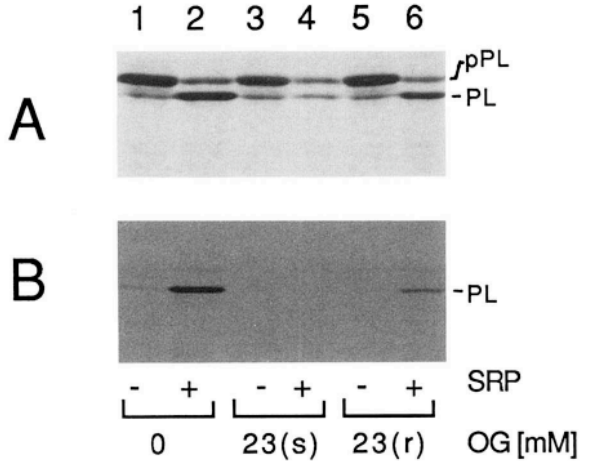

FIG. 5. **The recovery of translocation competent vesicles from detergent-treated microsomes requires the removal of OG.** *A*, translocation reactions (see "Experimental Procedures") were carried out in the presence of mock-treated microsomes (*lanes 1* and *2*), microsomes treated with 23 mM OG, *23(s)* (*lanes 3* and *4*), or microsomes reconstituted after treatment with 23 mM OG, *23(r)* (*lanes 5* and *6*) (see "Experimental Procedures"). OG was included at 5 mM in the reactions shown in *lanes 1* and *2*. SRP was included at 10 nM where indicated. The precursor protein, preprolactin (*pPL*), and processed prolactin (*PL*) are indicated. *B*, an aliquot of each reaction shown in *B* was treated with protease K before being prepared for SDS-PAGE.

between the nascent chain and the membrane components involved in translocation.

When the translations were subjected to digestion with protease K, the processed prolactin produced when reconstituted membranes and SRP were included in the reaction was protected from digestion (Fig. 5*B*, *lanes 5* and *6*). However, as expected, when detergent-saturated membranes were used, no such protease protection was observed (Fig. 5*B*, *lanes 3* and *4*). Similar results were obtained when membranes were treated with 24 or 25 mM OG (data not shown). In order to rule out the possibility that the small amount of OG present in the reactions containing detergent-saturated membranes inhibited translocation, we performed the translocation assays with mock-treated membranes in the presence of the same concentration of detergent (5 mM OG final). This concentration of detergent had no effect on translation or on translocation (Fig. 5, *lanes 1* and *2*). Taken together, these results suggest that translocation competent vesicles were not present in the detergent extract and that translocation competent vesicles form from detergent-disrupted membranes that reconstitute after detergent removal.

DISCUSSION

We have developed a method for reconstituting sealed vesicles from partially solubilized microsomal membranes. We have demonstrated that although partially solubilized microsomes are incompetent for translocation, reconstituted vesicles are translocation competent. Translocation by the reconstituted vesicles is SRP-dependent, indicating that nascent preprolactin chains are cotranslationally targeted to the membranes via an interaction between SRP and its receptor. Furthermore, no translocation is observed if preprolactin is added to reconstituted membranes post-translationally (not shown). Thus, we believe that in addition to signal peptidase (6) and SRP receptor (4, 5), all other components required for translocation are active after partial solubilization and reconstitution.

In addition to preprolactin, prepro-α-factor, the precursor for the yeast pheromone α-factor, was used as a substrate for translocation by microsomes reconstituted after treatment with 23 mM OG. Prepro-α-factor contains three asparagine residues which become glycosylated upon translocation (23). However, although prepro-α-factor was translocated across reconstituted membranes, as determined by cleavage of its signal sequence, no glycosylation of the translocated protein was detected (data not shown). Thus, disruption of the membrane by OG renders the microsomal vesicles inactive for glycosylation. This result is in agreement with the findings of Rothman *et al.* (24) who showed that the ability of microsomal vesicles to glycosylate the glycoprotein of vesicular stomatitis virus in an *in vitro* translocation reaction decreased when vesicles were reconstituted after treatment with increasing amounts of Triton X-100 relative to vesicle concentration.

We were able to reconstitute vesicles from microsomes treated with up to 25 mM OG. At this detergent concentration most of the lumenal proteins are released from the microsomes. By Western blotting we detect <2% of immunoglobulin heavy chain binding protein (21) in reconstituted membranes after extraction with 25 mM OG (data not shown). Thus, it seems unlikely that any lumenal proteins play an active role in translocation, although formally this possibility cannot be ruled out by our experiments. Our findings are in agreement, however, with Bulleid and Freedman (25) who reported that translocation activity can be recovered from microsomal membranes alkali-treated at pH 9. This treatment does not perturb the integrity of the lipid bilayer *per se*, but leads to the release of lumenal contents from the microsomes, presumably by causing the microsomal vesicles to open transiently (26).

When microsomes were treated with 23 mM OG, about 30% of the translocation activity of control microsomes were reconstituted. The percent of activity reconstituted decreased with increasing OG concentration, and so far we have been unable to recover activity when microsomes were treated with detergent concentrations in excess of 25 mM OG. Perhaps at higher detergent concentrations some of the lipid that is solubilized forms into lipid micelles and is excluded from protein-containing bilayers when the detergent is removed. Thus, upon detergent removal protein-containing bilayers might not reform into sealed vesicles. Alternatively, the higher detergent concentration may affect the translocon itself. Our finding that detergent-treated membranes display no SRP-dependent cleavage supports this second view. We would expect that if all the components of the translocon were still in contact with one another, then SRP-dependent targeting followed by efficient processing by signal peptidase would occur even in the absence of sealed vesicles.

Our ability to partially solubilize and then reconstitute microsomal vesicles provides a first step toward achieving reconstitution from completely solubilized vesicles. In addition, it may provide a way to incorporate membrane proteins that have been completely solubilized into partially solubilized microsomal membranes. Thus, it may now be possible to complement biochemically inactivated membrane proteins with solubilized active components.

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Note Added in Proof—Recently, similar results were reported (Yu, Y., Zhang, Y., Sabatini, D. D., and Kreibich, G. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 9931–9935).

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