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Site-specific antibodies against the PrlA (SecY) protein of *Escherichia coli* inhibit protein export by interfering with plasma membrane binding of preproteins

(synthetic peptides/Fab of IgG/cell-free translation/MalE and LamB/inverted vesicles)

MAKOTO WATANABE AND GÜNTER BLOBEL

Laboratory of Cell Biology, Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10021

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ABSTRACT **Genetic evidence indicates that the PrlA (SecY) protein of *Escherichia coli* functions as a membrane integrated signal sequence receptor in protein "export"—i.e., in protein translocation across (or integration into) the plasma membrane. We have raised antibodies in rabbits against two synthetic peptides representing the hydrophilic N- or C-terminal region of PrlA. Using these antibodies as probes in cell fractionation experiments, we confirm that PrlA is an integral membrane protein of the plasma membrane of *E. coli*. Fab fragments prepared from each of the two antisera specifically inhibit protein export by interfering with the binding of preproteins to the plasma membrane. Inhibition of preprotein binding and export by Fab fragments was shown in a cell-free translocation system: precursors for LamB (an integral membrane protein) and for a truncated form of MalE (a periplasmic protein) were first synthesized in a membrane-depleted *E. coli*-derived cell-free translation system followed by posttranslational incubation with inverted vesicles derived from the plasma membrane of *E. coli*. Our data thus indicate that the N and C termini of PrlA are exposed to the cytoplasm. We discuss the possibility that the transmembrane segments of PrlA could be arranged in the lipid bilayer in a cylindrical fashion, thereby delimiting a protein conducting channel, with a signal sequence binding domain represented, at least in part, by the N and C termini of PrlA. Such a channel would also contain a "stop-transfer" sequence binding domain that in response to a stop-transfer sequence would open the cylindrical channel to the lipid bilayer and permit displacement of the polypeptide from the channel to the lipid bilayer, resulting in membrane integration.**

Genetic approaches have identified several genes that function in protein "export"—i.e., in protein translocation across or integration into the plasma membrane of *Escherichia coli* (for review, see refs. 1 and 2). The products of these genes have been proposed to be components of the *E. coli* protein translocation machinery.

prlA is a member of this group of genes. It has been discovered as a suppressor of signal sequence mutations (3). Certain alleles of *prlA* have been shown to restore export of proteins containing defective signal sequences without affecting the translocatability of proteins containing "wild-type" signal sequences (4). The PrlA protein has therefore been proposed to function in signal sequence recognition. After the discovery of *prlA*, a gene termed *secY* was identified. A temperature-sensitive allele of *secY* conferred a temperature-sensitive phenotype for protein translocation and growth at the restricted temperature (5). Expression of *secY* was found to be essential for protein translocation (6). *SecY* (7) as well as *prlA* (8) have been shown to be located at the promoter-distal part of the *spc* ribosomal protein operon of *E. coli* and to represent one and the same gene.

The sequence of the *prlA* (*secY*) gene has been established (9). From the deduced amino acid sequence (9) and from N-terminal sequence analysis of the overproduced protein (10), it is known that PrlA consists of 443 amino acids with a calculated mass of 49 kDa. Hydropathy analyses showed that PrlA contains at least eight hydrophobic regions that could serve as membrane spanning segments. On the bases of its abnormal electrophoretic mobility, its partitioning into the nonionic detergent Nonidet P-40, and cell fractionation data, PrlA has been proposed to be an integral membrane protein of the *E. coli* plasma membrane (11).

Using a cell-free *E. coli*-derived translocation system, Fandl and Tai (12) have recently demonstrated that inverted vesicles from a temperature-sensitive *secY* mutant lose their translocation competence when exposed to the nonpermissive temperature.

In this paper, we present results with antibodies to two synthetic peptides that correspond to the N or C regions of PrlA. We show that Fab fragments of IgG against the N- or C-terminal domain of PrlA inhibit protein export in an *E. coli* cell-free system by interfering with binding of preproteins to inverted vesicles (INVs) derived from the *E. coli* plasma membrane.

MATERIALS AND METHODS

Materials. [^{35}S]Methionine (1000 Ci/mmol; 1 Ci = 37 GBq) and ^{125}I-labeled protein A (^{125}I-protein A) (7.2 μCi/μg) were from New England Nuclear. Antisera against LamB and MalE protein were generously provided by S. Benson (Princeton University) and B. A. Rasmussen (Princeton University), respectively.

Synthesis of Peptides. Peptides corresponding to the N- or C-terminal region of PrlA (see Fig. 1) were generously synthesized by R. Lerner (Research Institute Scripps Clinic) using solid-phase methods (13). An additional cysteine for the purpose of coupling to the protein carrier was added to the sequence (see Fig. 1).

Coupling the Synthetic Peptides to Carrier Protein. Peptides were coupled to the carrier protein keyhole limpet hemocyanin (KLH) through their cysteine residues with *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) as coupling reagent (14). KLH (25 mg) was dissolved into 750 μl of 10 mM sodium phosphate buffer (pH 7.2) and dialyzed against the same sodium phosphate buffer for 3 hr at 4°C. The dialyzed KLH solution was centrifuged for 5 min at 10,000 × *g* to remove undissolved material and the volume was

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Abbreviations: INVs, inverted vesicles derived from the *Escherichia coli* plasma membrane; KLH, keyhole limpet hemocyanin; MBS, *m*-maleimidobenzoyl-*N*-hydroxysuccinimide; TEA, triethanolamine; BSA, bovine serum albumin.

adjusted to 1.25 ml. To 1 ml of this KLH solution, 275 μl of 10 mM sodium phosphate buffer (pH 7.2) and 425 μl of MBS (6 mg/ml) in dimethylformamide were added. The reaction mixture was stirred for 30 min at room temperature and passed through Sephadex G-25 equilibrated with 50 mM sodium phosphate buffer (pH 6.0). Peak fractions were pooled by measuring A_{280}. To 0.75 ml of KLH-MBS (6 mg/ml) was added 1 ml of peptide solution (5 mg/ml) [in 50 mM sodium phosphate buffer (pH 6.0)], and the pH was adjusted to 7.0–7.5 with 1 M NaOH. The mixture was stirred for 3 hr at room temperature and stored at −20°C until used for immunization.

Preparation of Anti-peptide Antibodies. New Zealand White rabbits were injected subcutaneously at four sites with 240 μg of peptide-coupled KLH emulsified with complete Freund's adjuvant (1:1) for the primary injection. Subsequent injections of antigen (240 μg per rabbit) emulsified with incomplete Freund's adjuvant were at 2-week intervals. Sera were collected 1 week after the third injection.

Preparation of Fab Fragments. IgG was isolated from antisera by ammonium sulfate precipitation, followed by DEAE-Sephadex chromatography (15). Fab fragments were prepared from IgG according to Garvey *et al.* (16). IgG was digested with papain and dialyzed against 0.01 M acetate buffer (pH 5.5). The dialyzed sample was applied to a CM-cellulose column and elution was carried out by the stepwise addition of 0.05, 0.1, 0.225, and 0.45 M sodium acetate (pH 5.5). The fraction eluted by 0.05 M buffer was collected, concentrated by a Millipore immersible CX-10 ultrafiltration unit, and passed through a Sephadex G-25 column to change the buffer to 0.05 M triethanolamine (TEA)-OAc (pH 7.5). The concentration of Fab fragments was determined by the bicinchoninic acid protein assay (Pierce) with bovine serum albumin (BSA) as standard.

Subcellular Fractionation. Subcellular fractionation was done according to Schnaitman (17). *E. coli* strain MRE 600 cells were grown to A_{600} = 1.8 in a medium containing (per liter) 5.6 g of KH_2PO_4 (anhydrous)/28.9 g of K_2HPO_4 (anhydrous)/10 g of yeast extract/10 g of bactotryptone/10 g of glucose. Cells were resuspended (1 g/ml) in buffer A (50 mM TEA-OAc, pH 7.5/250 mM sucrose/1 mM EDTA/1 mM dithiothreitol/0.5 mM phenylmethylsulfonyl fluoride, and passed through a French press twice at 4000 psi (1 psi = 6.9 kPa). The cell lysate was freed of unbroken cells and cell debris (10 min at 3000 × *g*) and the resulting supernatant (homogenate) was centrifuged for 2 hr at 150,000 × g_{av}. The pellet (total membranes) derived from 1 liter of culture was resuspended in 2 ml of buffer A by using a loosely fitting Dounce homogenizer and 0.5 ml of this suspension was layered on top of a sucrose gradient in buffer A according to Schnaitman (17). After centrifugation, the gradient was divided into four fractions; top, plasma membrane, intermediate, and outer membrane fraction. Aliquots containing 100 μg of protein of homogenate, supernatant, total membrane, and each of the four fractions were analyzed by $NaDodSO_4$/PAGE and immunoblotting (see below). INV of the plasma membrane used for posttranslational translocation (see below) were prepared as described (18).

Posttranslational Translocation Assay. mRNA (200 ng) for LamB or a truncated form of MalE lacking 49 C-terminal residues but containing 7 additional residues not found in the original sequence and a termination codon (unpublished data) and referred to as MalE* was translated in an *E. coli* cell-free translation system depleted of plasma membranes as described (18). After translation, the mixture was centrifuged for 30 min at 30 psi in the Airfuge rotor A100/18 to sediment ribosomes. The postribosomal supernatant was passed twice through Sephadex G-25 equilibrated with 40 mM TEA-OAc, pH 7.5/140 mM KOAc/11 mM $Mg(OAc)_2$/0.8 mM spermidine/0.1 mM EDTA. The G-25 postribosomal supernatant was supplemented with 5 mM ATP, 0.5 A_{280} unit of high salt washed INV per ml, and, where indicated, Fab fragments and peptides. After incubation for 60 min at 25°C, the reaction mixture was treated with proteinase K (300 μg/ml) on ice for 30 min. The digestion was stopped by the addition of 10 mM phenylmethylsulfonyl fluoride and an equal volume of 20% trichloroacetic acid. The precipitate was processed for $NaDodSO_4$/PAGE. In some cases (see Fig. 5), membrane sedimentation was performed as described (18).

$NaDodSO_4$/PAGE. Procedures for $NaDodSO_4$/PAGE and subsequent autoradiography of dried slab gels were as described (19). The samples for $NaDodSO_4$/PAGE were precipitated with an equal volume of 20% trichloroacetic acid, and the precipitate was resuspended in 0.5 M Tris base containing 6.25% $NaDodSO_4$ by sonication and subsequent incubation at 37°C for 1 hr. Thereafter, the samples were reduced with dithiothreitol (100 mM) at 37°C for 1 hr or, in one case (see Fig. 2, lane 10), at 100°C for 5 min. Acrylamide gradient gels (10–15%) were used throughout except in one case (lane 11), where a 22.5% acrylamide gel was used.

To quantitate the radioactivity in a specific polypeptide species in the dried gel, we used a Beta Scanning System (Automated Microbiology Systems, San Diego, CA).

Immunoblotting. After $NaDodSO_4$/PAGE, proteins were electrophoretically blotted onto nitrocellulose in 20 mM Tris base/150 mM glycine/20% methanol at 80 V for 20–24 hr. The nitrocellulose blots were incubated for 1 hr in 3% BSA/140 mM NaCl/10 mM KPO_4, pH 7.5. Blots were then washed with distilled water and incubated overnight with anti-peptide antisera diluted 1:50 in buffer B (1% BSA/140 mM NaCl/10 mM KPO_4, pH 7.5/0.1% Triton X-100/0.02% $NaDodSO_4$), washed with buffer B without antisera and BSA, incubated overnight with ^{125}I-protein A diluted 1:2500 in buffer B, washed with 560 mM NaCl/10 mM KPO_4, pH 7.5/0.5% Triton X-100/0.1% $NaDodSO_4$, dried, and exposed to Kodak XAR film.

RESULTS

Synthetic Peptides Corresponding to N- and C-Terminal Regions of the PrlA Protein. A hydropathy analysis of the primary protein sequence using the algorithm of Eisenberg *et al.* (20) suggested that PrlA might contain as many as eight hydrophobic segments (Fig. 1, open boxes) that would be long enough to span the membrane and two hydrophobic segments (stippled boxes) that would be too short to span the membrane as an α-helix. Two peptides were synthesized that corresponded to hydrophilic regions at the N or C terminus of PrlA (bars). These hydrophilic segments of PrlA were chosen for immunization because they are likely to be exposed to the hydrophilic environment of the membrane, although no information was available at that time as to whether these domains are exposed on the cytoplasmic or on the periplasmic side of the plasma membrane (see below).

Detection of PrlA Protein Expressed by Chromosomal Gene. *E. coli* cells of the MRE 600 strain were broken by passage through a French press. A fraction representing the plasma membranes was prepared. Equal amounts of protein of both the homogenate (Fig. 2, lane H) and the plasma membrane fraction (lane PM) were analyzed by $NaDodSO_4$/PAGE and then either stained with Coomassie blue (Fig. 2 *Left*) or blotted onto nitrocellulose sheets and probed with preimmune sera or antisera against the synthetic peptides (Fig. 2 *Center*). Both the anti-N and the anti-C antisera reacted strongly with a 35-kDa protein present in the cell homogenate and the plasma membrane fraction.

Ito (6) had previously reported that PrlA aggregates when boiled in $NaDodSO_4$ and that it migrates abnormally fast in gels that are not highly crosslinked. In agreement with Ito's data, there was no reactivity with a 35-kDa protein when the

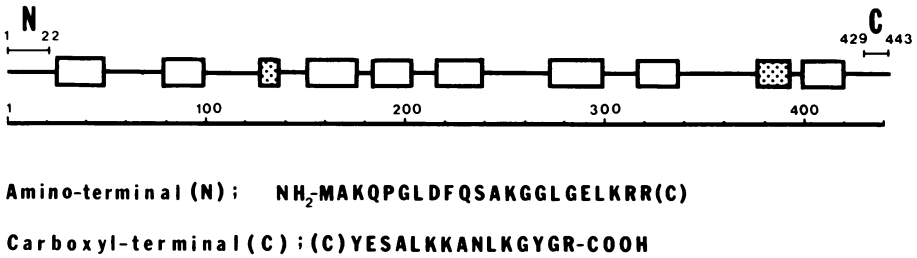

FIG. 1. Chemically synthesized peptides, their amino acid sequence (single-letter code), and their location along the PrlA protein. Regions of the protein selected for peptide synthesis are marked by lines and labeled N and C. Both peptides contain an additional cysteine (C) residue not found in the primary sequence but used for coupling with KLH. The hydropathy program used for the computation analysis is based on the algorithm of Eisenberg *et al.* (20). Open boxes, stretches of hydrophobic amino acid residues long enough to span the membrane; stippled boxes, stretches of hydrophobic amino acid residues but not long enough to span the membrane.

sample was boiled in $NaDodSO_4$ prior to electrophoresis (Fig. 2, lane 10). Moreover, an anti-C reactive polypeptide, now migrating at 42 kDa, was detected when the plasma membrane fraction was electrophoresed in a more highly crosslinked gel (lane 11). Taken together, these data show that the polypeptide detected by the anti-N and the anti-C antisera is PrlA. From a comparison of band intensities in the homogenate and plasma membrane fractions, it is clear that PrlA is enriched in the plasma membrane fraction (Fig. 2).

Subcellular Localization of PrlA. To determine the location of PrlA, we fractionated MRE 600 cells according to Schnaitman (17) (Fig. 3). A homogenate (lane H) was subjected to differential centrifugation to yield a supernatant (lane S) and crude membrane fraction (lane M). The latter was resuspended and layered on top of a sucrose gradient. After centrifugation, the gradient was divided into four fractions: top, plasma membrane, intermediate, and outer membrane fractions. An aliquot of each fraction was then analyzed by $NaDodSO_4$/PAGE. The polypeptides were either stained (Fig. 3*A*) or blotted onto nitrocellulose and then probed with anti-C antisera (Fig. 3*B*), anti-LamB antisera (Fig. 3*C*), or anti-MalE antisera (Fig. 3*D*). Most of PrlA sedimented with the crude membrane fraction (Fig. 3*B*, lane S versus lane M) and, after sucrose gradient fractionation of the crude membrane fraction, with the plasma membrane fraction (Fig. 3*B*, lanes 4–7). The efficacy of cell fractionation was confirmed by the distribution of LamB and MalE. LamB (an integral membrane protein of the outer membrane) was found in the crude membrane fraction (Fig. 3*C*, lane 3) and subfractionated predominantly into the outer membrane fraction (lane 7) with some LamB sedimenting also in a fraction intermediate between the plasma membrane and the outer membrane fraction (lane 6). The plasma membrane fraction was free of

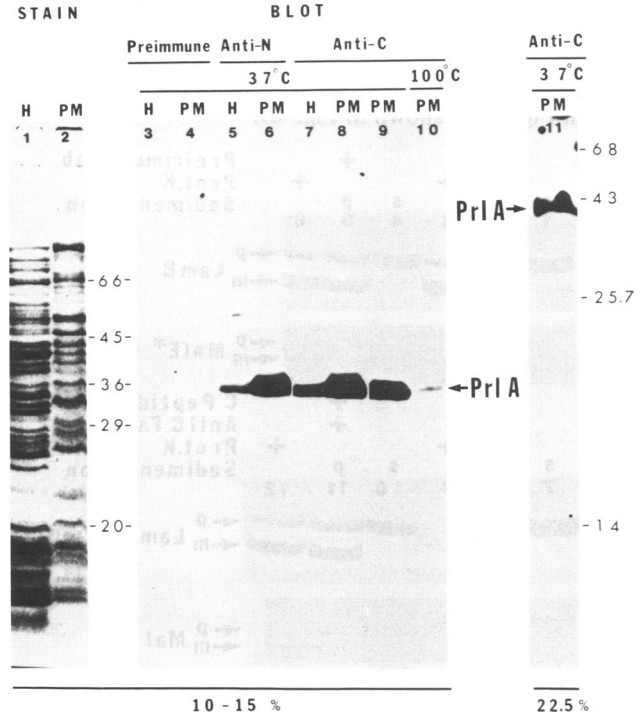

FIG. 2. Identification of PrlA by immunoblot using anti-peptide antisera. A homogenate (lanes H) or plasma membrane fraction (lanes PM), 100 μg protein each, was analyzed by $NaDodSO_4$/PAGE. Polypeptides were either stained (lanes 1 and 2) or blotted onto nitrocellulose and probed with preimmune or anti-peptide antisera (lanes 3–11). Sample preparation was carried out at 37°C or 100°C as indicated. The acrylamide concentration is indicated at the bottom. Numbers to the right of lanes 2 and 11 indicate kDa of marker proteins. PrlA is indicated by an arrow.

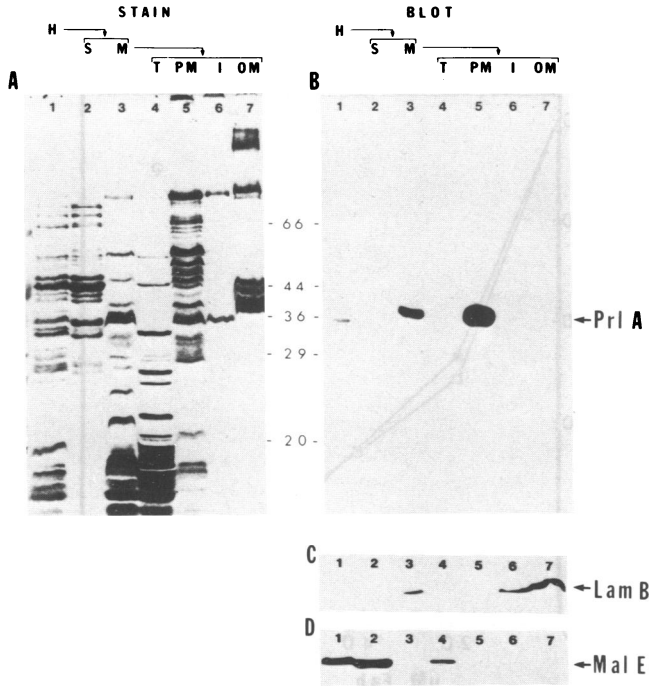

FIG. 3. Subcellular localization of the PrlA. A homogenate (lanes H) was centrifuged (2 hr at 150,000 × *g*) to yield a supernatant (lanes S) and membrane (lanes M) fraction. The latter was then subjected to sucrose gradient centrifugation and the gradient was divided into a top (lanes T), plasma membrane (lanes PM), intermediate (lanes I), and outer membrane (lanes OM) fraction. Aliquots (100 μg of protein) were analyzed by $NaDodSO_4$/PAGE. Polypeptides were either stained (*A*) or blotted onto nitrocellulose (*B–D*). Blots were probed with either anti-C antiserum (*B*), anti-LamB antiserum (*C*), or anti-MalE (maltose binding protein) antiserum (*D*). The LamB is an outer membrane protein, while the MalE is a periplasmic protein. PrlA, LamB, and MalE are indicated by arrows. Markers are the same as in Fig. 2.

detectable LamB and therefore of outer membranes. MalE (a soluble protein in the periplasmic space) was found primarily in the soluble fraction (Fig. 3*D*, lane 2). The small amount present in the crude membrane fraction sedimented at the top of the gradient (lane 4). Taken together, these cell fractionation data demonstrate that PrlA is associated exclusively with the plasma membrane. To examine whether PrlA is an integral membrane protein as predicted from the analysis of hydrophobicity (Fig. 1), we incubated the plasma membrane fraction either at alkaline pH (pH 11.5–12.5), with high salt (1 M KOAc) or with EDTA (25 mM), and separated the extracted material from the membrane by differential centrifugation. Neither incubation procedure extracted PrlA, indicating that it is an integral membrane protein (data not shown).

Fab Fragments of Anti-N or Anti-C IgG Inhibit Integration of Pre-LamB. Based on genetic evidence, PrlA has been proposed to function in signal sequence recognition (1, 3, 4, 6, 11). If the N- and C-terminal domains of PrlA would be exposed to the cytoplasm (i.e., on the surface of INV) and if they would play a role in signal sequence recognition, our anti-N or anti-C antibodies might conceivably react with these domains and thereby inhibit translocation (integration) in a cell-free system.

We therefore prepared IgG from preimmune sera and the anti-N and the anti-C antisera and tested them in a posttranslational translocation system. The latter consisted of an *E. coli* translation system that was programmed with *in vitro* transcribed LamB mRNA and was posttranslationally incubated with INV. We had previously demonstrated that LamB, although its final location is in the *E. coli* outer membrane, is integrated into INV suggesting that, *in vivo*, this protein is first integrated into the plasma membrane and subsequently transported to the outer membrane (18). In the presence of both the anti-N or the anti-C (but not the preimmune) IgG, posttranslational integration of pre-LamB into INV is inhibited (data not shown).

As inhibition of integration could have resulted from aggregation of PrlA in the plane of the membrane by the bivalent IgGs, we prepared monovalent Fab fragments and tested them in the posttranslational translocation system (Fig. 4). Only membrane integrated chains [i.e., chains resistant to proteinase K digestion and consisting of both precursor and mature forms of LamB (18)] are shown in Fig. 4*A*. By comparing the amount of total LamB chains in the translocation reaction to the amount of the protease-protected forms, we estimated that ≈50% of LamB chains were integrated (Fig. 5). In the absence of any added Fab fragments (data not shown), there was a similar amount of integrated LamB chains as in the presence of 80 μM preimmune Fab fragments (Fig. 4*A*). However, in the presence of increasing amounts of anti-N or anti-C Fab fragments, there was an increasing inhibition of integration. Moreover, inhibition at the highest concentration of either anti-N or anti-C Fab fragments was effectively blocked by competition with the corresponding peptide, indicating that inhibition by Fab fragments was indeed specific. A quantitative analysis of the data in Fig. 4*A* is shown in Fig. 4*B*.

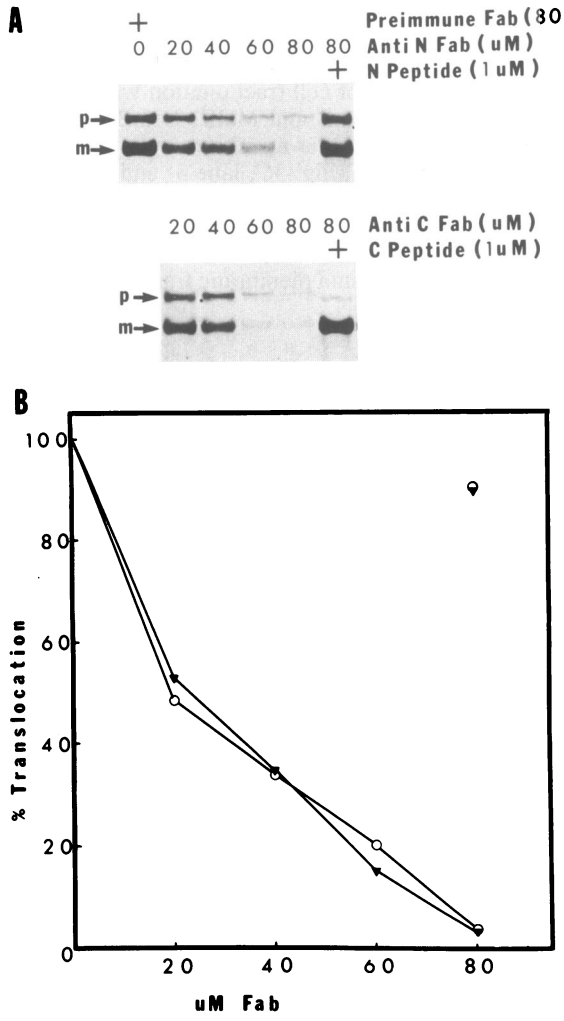

FIG. 4. Fab fragments of anti-N and anti-C IgG inhibit *in vitro* integration of LamB into inverted vesicles. mRNA for LamB was translated in an INV depleted cell-free translation system of *E. coli*. Posttranslational incubation with INV and, when indicated, with various Fab fragments and peptides was followed by another incubation with proteinase K. Only membrane-integrated chains composed of either precursor (p) or mature (m) LamB protein are protected from proteolysis (18). (*A*) Autoradiographs. (*B*) Quantitation of the data in *A*. In *B*, the anti-N Fab fragments data are indicated by open circles and the anti-C Fab fragments data are shown by closed triangles. Open circle and closed triangle in the upper right corner of *B* represent data where Fab fragments and corresponding peptides were assayed together (see *A*).

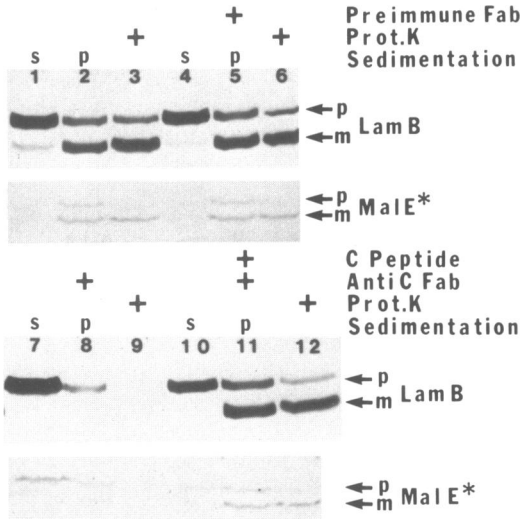

FIG. 5. Fab fragments of anti-C IgG inhibit MalE* and LamB export to INV by interfering with the binding of their precursors to INV. mRNA for MalE* or LamB was translated in an *E. coli* system and posttranslational incubation with INV was done as in Fig. 4. Aliquots were then subjected to proteinase K digestion or to centrifugation to yield a pellet fraction (lanes p) containing INV and INV bound precursor or mature forms and a supernatant fraction (lanes s) containing unbound precursors. Where indicated, Fab fragments were used at a concentration of 80 μM and C peptide was used at a concentration of 1 μM. For details, see Fig. 4 and *Materials and Methods*.

Inhibition of Export by Fab Fragments Results from Interference in the Binding of Precursors to INV. To determine whether the observed inhibition of integration by the anti-N or anti-C Fab fragments resulted from interference in the binding of precursors to INV, we used centrifugation to separate membrane-bound from unbound precursors (Fig. 5). Indeed, only a small amount of precursors of MalE* and LamB bound to INV (compare lanes 8 and 7) and none was exported (lane 9) in the presence of anti-C Fab fragments. Inhibition of binding (and export) by anti-C Fab fragments was reversed when C peptide was also present (lanes 10–12). In the presence of both C peptide and anti-C Fab fragments, the amount of exported chain was similar to that observed in the absence of Fab fragments (lanes 1–3) or to that in the presence of Fab fragments prepared from preimmune sera (lanes 4–6). Results similar to those with the anti-C Fab fragments (lanes 7–12) were obtained with anti-N Fab fragments (data not shown).

DISCUSSION

Our results suggest that precursors of secretory and integral membrane proteins bind to PrlA of the *E. coli* plasma membrane prior to their translocation across or integration into the plasma membrane. This conclusion is based on data obtained with antibodies, one against a peptide representing the N-terminal region of PrlA and the other representing its C-terminal region. In a cell-free translocation (integration) system consisting of an *E. coli* translation system supplemented with INVs derived from the *E. coli* plasma membrane, Fab fragments prepared from IgG against either of these two peptides inhibited integration of LamB or translocation of MalE*. Inhibition was observed to result from interference by the Fab fragments in the binding of the precursors to the membrane and is specific as it is completely reversed in the presence of the corresponding peptides. Taken together, these data indicate that binding of the precursor to PrlA is required for subsequent translocation across or integration into the membrane. As genetic data suggest that PrlA functions as a signal receptor (1), binding of the precursors to PrlA is likely to occur via their signal sequence.

Our finding here that Fab fragments against N- or C-terminal peptides of PrlA inhibit precursor binding to INV indicate that both N- and C-terminal regions of PrlA are exposed to the cytoplasm (PrlA therefore must contain an even number of transmembrane segments; see also ref. 21) and that both regions might be involved in signal sequence recognition, either directly or indirectly. If the transmembrane segments of PrlA were arranged in the membrane in a cylindrical fashion, the N- and C-terminal regions might form a distinct cytoplasmically exposed domain. If this domain would function in signal sequence recognition, Fab fragments would interfere with precursor binding by blocking access of the signal sequence to this domain. Alternatively, the N- and C-terminal domains of PrlA may not constitute a signal sequence recognition domain. Binding of Fab fragments to these regions of PrlA might interfere indirectly—e.g., by steric hindrance—with signal sequence binding to another domain.

A cylindrical arrangement of the 8 (or perhaps 10) transmembrane segments of PrlA could also provide for a central channel across the membrane through which chain translocation could occur (11, 21). However, unlike ion conducting channels that need to open and close in only one dimension, polypeptide conducting channels would have to open and close in two directions, across the membrane and to the lipid bilayer. Opening to lipid bilayer is necessary because transmembrane segments of integral membrane protein would have to be displaced from the channel into the lipid bilayer. In a cylindrical arrangement, putative contact sites between the N- and C-terminal transmembrane segments of PrlA might constitute a site for a reversible lateral opening of the channel to the lipid bilayer—e.g., in response to a stop transfer sequence.

Thus, in one scenario, a signal sequence, by virtue of its binding to the cytoplasmically exposed signal sequence recognition domain of PrlA (see above), could trigger opening of the channel in one dimension, across the plasma membrane, initiating the process of chain conductance. In the case of secretory protein, the process would go to completion followed by closure of the channel until it would be reopened by the signal sequence of another secretory protein. In case of membrane proteins, a signal sequence would also open the channel in one dimension and chain conductance would proceed until the appearance in the channel of a stop transfer sequence. The latter would open the channel in a second dimension, to the lipid bilayer, and thereby halt chain conductance and yield integration of the domain into the lipid bilayer.

Alternatively, a protein conducting channel might be formed by several PrlAs (homooligomeric channel) or by an association of PrlA with other integral membrane proteins (heterooligomeric channel).

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