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Inhibition of Preprotein Translocation and Reversion of the Membrane Inserted State of SecA by a Carboxyl Terminus Binding MAb†

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ABSTRACT: SecA is the peripheral subunit of the preprotein *translocase* of *Escherichia coli*. SecA consists of two independently folding domains, i.e., the N-domain bearing the high-affinity nucleotide binding site (NBS-I) and the C-domain that harbors the low-affinity NBS-II. ATP induces SecA insertion into the membrane during preprotein translocation. Domain-specific monoclonal antibodies (mAbs) were developed to analyze the functions of the SecA domains in preprotein translocation. The antigen binding sites of the obtained mAbs were confined to five epitopes. One of the mAbs, i.e., mAb 300-1K5, recognizes an epitope in the C-domain in a region that has been implicated in membrane insertion. This mAb, either as IgG or as Fab, completely inhibits *in vitro* proOmpA translocation and SecA translocation ATPase activity. It prevents SecA membrane insertion and, more strikingly, reverses membrane insertion and promotes the release of SecA from the membrane. Surface plasmon resonance measurements demonstrate that the mAb recognizes the ADP- and the AMP-PNP-bound state of SecA either free in solution or bound at the membrane at the SecYEG protein. It is concluded that the mAb actively reverses a conformation essential for membrane insertion of SecA. The other mAbs directed to various epitopes in the N-domain were found to be without effect, although all bind the native SecA. These results demonstrate that the C-domain plays an important role in the SecA membrane insertion, providing further evidence that this process is needed for preprotein translocation.

SecA is the central component of the preprotein *translocase* in *Escherichia coli* (for reviews, see ref *1*, *2*). Preprotein *translocase* is a multimeric membrane protein complex that in addition to SecA consists of the integral membrane proteins SecY, SecE, and SecG as stable subunits (*3*−*5*), and SecD and SecF as accessory proteins (*6*, *7*). The dynamic distribution of SecA between the cytosol and the membrane is determined by the amount of integral *translocase* subunits present in the membrane and by growth conditions (*8*−*11*). The heterotrimeric SecYEG complex constitutes a high-affinity membrane binding site for SecA (*3*, *12*), and the SecA bound at these sites exposes a carboxy-terminal domain to the periplasmic face of the membrane (*8*). SecA may also directly bind to the phospholipids at the membrane surface that constitute a binding site with low affinity (*13*, *14*). SecA is required for the productive binding of preproteins to the *translocase* (*3*, *4*). It interacts with the signal sequence and with a part of the mature preprotein (*15*, *13*) but also binds SecB, a molecular chaperone that is specific for preprotein translocation (*3*). A key feature of

the SecYEG-bound SecA is its ATPase activity that is stimulated by preproteins (*13*). SecA couples the hydrolysis of ATP to the translocation of the preprotein across the membrane. SecA is a large protein of 102 kDa, and not only exists (*16*) but also functions (*17*) as a stable homodimer. Each of the protomers comprises two independently folding domains of similar size, which have been termed the amino (N) and the carboxy (C) terminal domains (*18*). The N-domain contains a high-affinity ($K_{D,ADP} \approx 0.15$ μM) nucleotide binding site (NBS-I),[1] whereas the C-domain contains a site that binds nucleotides with low affinity ($K_{D,ADP} \approx 340$ μM, NBS-II) (*19*, *20*). Both NBSs are able to bind ATP independently *in vitro*, whereas mutants in either site inhibit the translocation ATPase activity of SecA completely, indicating that both sites are essential for and act in a concerted manner in the SecA-mediated translocation (*20*−*22*). During translocation, preproteins are pushed across the membrane in steps of about 2−3 kDa (*23*). This process is thought to be driven by nucleotide-modulated cycles of membrane inserted SecA (*24*, *25*, *14*). In the presence of preprotein, the binding of ATP (or the nonhydrolyzable ATP analog AMP-PNP) drives the insertion of SecA into SecA-depleted membranes in a SecYEG-dependent fashion. This gives rise to a number of proteinase resistant SecA fragments

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[1] Abbreviations: AMP-PNP, adenosine 5′-(β,γ-imidotriphosphate); BSA, bovine serum albumin; DTT, dithiothreitol; ELISA, enzyme linked immunosorbent assay; Fab, ab fragment from IgG; IMV, inverted inner membrane vesicles; mAb, monoclonal antibody; NBS, nucleotide binding site; TBS, Tris-buffered saline; SPR, surface plasmon resonance.

using uniformly ^{35}S-labeled SecA (*26, 14*) or a 30-kDa carboxy-terminal fragment using ^{125}I-SecA (*24–26*; Figure 1). Based on the observation that ATP hydrolysis stimulates the de-insertion of the 30-kDa ^{125}I-SecA fragment, and that a second ATP molecule has to be hydrolyzed to release the SecA from the membrane, it has been suggested that the de-insertion of SecA is an essential part of the preprotein translocation cycle (*24, 25*). However, in ^{35}S-SecA-reconstituted membranes, about 50–60% of the ^{35}S-SecA can be released by unlabeled SecA independent of protein translocation and the remaining SecA seems to be stably inserted and fully functional in preprotein translocation (*14*), suggesting, that the de-insertion of SecA may not to be a prerequisite for preprotein translocation.

The mechanistic details, how the nucleotide-induced conformational changes in SecA are coupled to preprotein translocation, are only poorly understood. Differential scanning calorimetry and dynamic light scattering experiments with soluble SecA (*18*) indicate that the AMP-PNP-bound state of SecA has a more extended conformation than the ADP-bound state. The ADP-bound state may resemble the membrane de-inserted, surface-bound state of SecA, whereas the more extended AMP-PNP-bound state possibly corresponds to the membrane inserted conformation. Binding of nucleotides to NBS-I changes the interaction between the N- and C-domains (*18*), possibly by closing and opening of a cleft between both domains. To provide further information about the functions of the C- and N-domains, we have raised monoclonal antibodies against specific parts of SecA. One mAb recognizes an epitope that is confined to the 30-kDa carboxyl-terminal region of SecA that has been shown to insert into the membrane (*26*). This mAb efficiently inhibits *in vitro* translocation and blocks the SecA translocation ATPase activity. Inhibition is due to the prevention of SecA membrane insertion and, more strikingly, the reversal of the membrane inserted state of SecA. It is concluded that the mAb inhibits preprotein translocation by interfering with a membrane inserted conformation of SecA.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth Media. Unless indicated otherwise, strains were grown in Luria Bertani (LB) broth or on LB-agar supplemented with 50 μg of ampicillin/mL, 0.5% (w/v) glucose, or 1 mM isopropyl 1-thio-β-D-galactopyranoside, as required. SecYEG overproduction was done in SF100 freshly transformed with pET340 (YEG[+]), SecA in JM109 and NO2947 containing pMKL18, SecB in NO2947 (Table 1) containing pAK330 (*27*), and proOmpA in *E. coli* strain MM52 transformed with pTAQpOA (gift of N. Nouwen).

Materials. SecA, SecB, and proOmpA were isolated as described (*28–30*, respectively). Purified SecA was labeled with carrier-free [125]I (Radiochemical Centre, Amersham, U.K.) to a specific activity of about 5×10^4 cpm/pmol according to the following procedure: SecA (100 μg) in buffer A (50 mM TrisHCl, pH 8.0, 50 mM KCl, 5 mM MgCl$_2$, and 10% glycerol) was transferred to a reaction vial coated with IODO-GEN Iodination Reagent (Pierce, Rockford, IL). The reaction was started by adding 2 μL of K[125]I (200 μCi), incubated for 15 min at room temperature, and terminated by transferring the mixture into a new reaction vial containing dithiothreitol (DTT) at a final concentration of 10 mM. Free iodine was removed by chromatography on a PD-10 Sephadex column (Pharmacia LKB Biotech AB, Uppsala, Sweden) which was prewashed with buffer A, containing 1 mM DTT.

Inverted inner membrane vesicles (IMV; *31*) were prepared from *E. coli* D10 and SF100 which was transformed either with pET324 (*8*) as a control or with pET340 (SecYEG[+]; Table 1). The fusion proteins *cro*-β-galactosidase–SecA-(323–706) and *cro*-β-galactosidase–SecA(149–323) were purified from strain POP2136 containing the *cI*ts857 repressor harboring the plasmids pET111 and pET135, respectively. Monoclonal antibodies were produced as hybridoma supernatants (MCA development b.v. Groningen, The Netherlands) or as ascites (Eurogentec, Gent, Belgium) and purified by protein G–Sepharose 4 Fast Flow affinity chromatography as recommended by the company (Pharmacia). Fab fragments were obtained using the ImmunoPure Fab preparation kit of Pierce as recommended. AMP-PNP was purified of contaminating ADP as described (*18*). Anti-mouse IgG Fcγ was from Pharmacia (Biosensor AB, Uppsala, Sweden). All chemicals were from Sigma (St. Louis, MO) if not stated otherwise.

Construction and Expression of the Fusion Gene lacZ-secA. A DNA fragment encoding a truncated SecA molecule was obtained by digestion of pMKL18 encoding wild-type SecA with *Sal*I–*Pst*I or with *Eco*RV-*Sal*I. The fragments were made blunt by T4-polymerase/exonuclease treatment and cloned in-frame with *lacZ* in the multiple cloning site of pEX1 and pEX2, respectively. The pEX plasmids contain a synthetic operon expressing a 117 000 Da *cro*-β-galactosidase hybrid protein under control of the P$_R$ promoter of bacteriophage λ (*32*). All DNA manipulation techniques were essentially done as described (*33*). The resulting constructs pET111 and pET135 were transformed into strain POP2136 and grown at 30 °C in LB. An overnight culture was diluted 1:100 in 200 mL of LB and grown at 30 °C for 2 h, after which the fusion protein expression was induced by a shift to 42 °C. After 3 h, the cells were harvested by centrifugation. The pellet was resuspended in Laemmli sample buffer (*34*) and boiled for 5 min to lyse the cells. Debris was removed by centrifugation at 14000*g*, and the lysate was loaded onto preparative 7.5% SDS–PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis, *34*). After electrophoresis, the gel was stained by 0.3 M CuCl$_2$, and a protein band of approximately 150 kDa was excised and electroeluted overnight at 90 V. The protein solution was washed with phosphate-buffered saline and concentrated by centrifugation in a 10 000 NMWL filter unit (Millipore, Bedford, MA). Protein concentration was determined as described (*35, 36*).

Immunization. At day 0, BALB/c mouse 322, 323, and 300 were immunized by injection intraperitoneally with 50 μg of the β-galactosidase–SecA(323–706) and β-galactosidase–SecA(149–323) fusion proteins and with purified wild-type SecA mixed with complete Freund's adjuvant, respectively. On day 54 and 90, the mice were injected intraperitoneally with a second 50 μg of protein with incomplete Freund's adjuvant. On day 104, a blood sample was taken from the mice to monitor the presence of SecA- and β-galactosidase-specific antibodies. After 110 days, the mice were boosted intravenously with 50 μg of purified wild-type SecA to activate B lymphocytes that produce SecA-specific antibodies. Three days later, lymphocytes were isolated from the spleen for fusion with SP20 myeloma cells by standard techniques (*37*).

Selection of MAbs. Hybridomas were selected on the basis of a strong positive reaction in an ELISA (enzyme linked immunoadsorbent assay) with purified wild-type SecA and with the corresponding fusion protein and a negative reaction with β-galactosidase. A second selection was carried out on the basis of a strong positive reaction with wild-type SecA in cell extract on immunoblot analysis. Hybridomas showing positive reactions of the desired specificity were cloned by the limiting dilution technique at a density of 3−5 cells/ well and recloned at a density of 0.3 cell/well. Selected cultures were grown in bulk, and the culture supernatant of selected hybridomas was used for the characterization of the mAbs. The immunoglobulin (Ig) subclasses of the mAbs were determined by ELISA with peroxidase-conjugated subclass-specific antisera (ITK Diagnostics B. V., Uithoorn, The Netherlands).

Epitope Mapping. The epitopes of the mAbs were deduced from their affinity for purified truncated SecA molecules in an ELISA assay and whole cell extracts containing β-galactosidase−SecA fusion proteins analyzed by immunoblotting. The amino- and carboxy-terminal truncated SecA molecules were constructed and purified as described previously (*38*). β-Galactosidase−SecA fusion proteins for the epitope mapping were constructed as follows. A *secA* fragment was obtained by digestion of pMKL18 encoding wild-type SecA with *Sal*I−*Kpn*I or *Sal*I−*Sna*BI. The fragments were made blunt by T4-polymerase/exonuclease treatment and cloned in frame with *lacZ* in the multiple cloning site of pEX1. The resulting constructs pET112 and pET113 were transformed into strain POP2136 and expressed as described (see above).

In Vitro Translocation. In vitro translocation of ³⁵S-labeled proOmpA was performed as described (*3*) with 20 μg/mL SecA, 32 μg/mL SecB, 2 mM ATP, 10 mM phosphocreatine, and 50 μg/mL creatine kinase in translocation buffer [50 mM HEPES−KOH, pH 7.6, 30 mM KCl, 2 mM DTT, 0.5 mg/mL bovine serum albumin (BSA), and 2 mM MgOAc]. ³⁵S-Labeled proOmpA was diluted 50-fold from a solution of 8 M urea and 50 mM Tris-HCl, pH 7.6, followed by the addition of *E. coli* IMV at a final protein concentration of 1.7 mg/mL or urea-treated IMV (0.2−0.5 mg/mL). Reactions were incubated for 30 min at 37 °C, and stopped by cooling on ice. Samples were treated with proteinase K (0.1 mg/mL) for 15 min on ice. Subsequently, the samples were precipitated with 6.7% (w/v) trichloroacetic acid, washed with acetone, and analyzed by SDS−PAGE and autoradiography.

SecA Translocation and Endogenous ATPase Assay. The translocation ATPase activity of SecA using urea-treated vesicles (*28*) was measured as described (*39*) except that the reactions were supplied with 32 μg/mL SecB. The endogenous SecA ATPase activity (i.e., in the absence of vesicles and preprotein) was determined in 50 μL of 50 mM TrisHCl, pH 8.0, 50 mM KCl, 5 mM MgCl₂, 1 mM DTT, 0.5 mg/mL BSA, 17 μg/mL SecA, and 1 mM ATP and incubated for 3 h at room temperature.

Insertion and De-insertion of Iodinated SecA. ¹²⁵I-SecA (16 nM) was incubated 30 min in translocation buffer supplemented with proOmpA, SecB, and urea-treated YEG⁺ IMV (200 μg/mL) in the presence or absence of mAb and AMP-PNP or ATP at 0 and 37 °C, respectively. The reaction was terminated by proteinase K digestion as described (*24*). The digests were analyzed by 12% SDS−PAGE and by phosphor imaging (Phosphor Imager, Molecular Dynamics,

Sunnyvale, CA) and quantitated using the program Image Quant V3.3 of the same company.

Membrane Binding of SecA. ¹²⁵I-SecA (16 nM) was incubated 20 min in translocation buffer supplemented with proOmpA, SecB, 1 mM AMP-PNP, and urea-treated YEG⁺ IMV (200 μg/mL) at 37 °C (*3*). To remove unbound SecA, the sample was centrifuged through 0.2 M sucrose in translocation buffer (10 min, 30 psi in a Beckman airfuge at 4 °C), and the membranes were resuspended in 80 μL of 1.25 × translocation buffer supplemented with 1.25 mM AMP-PNP. To these samples was added mAb IgG (66 nM) or SecA (200 nM) and the volume adjusted to 100 μL. Membrane-bound SecA was separated from soluble SecA by centrifugation through a sucrose cushion as described (see above). The ¹²⁵I-SecA in the supernatant and pellet were quantified in a gamma counter.

Surface Plasmon Resonance (SPR). Real time detection of antibody−antigen binding and kinetic analysis at 25 °C were performed using the BIAlite surface plasmon resonance based system (Pharmacia Biosensor). The principle and application of the system employing the method of surface plasmon resonance detection has been described by Karlsson *et al.* (*40*). MAb 300-1K5 (50 μg/mL in 10 mM sodium acetate, pH 4.8) was immobilized to the sensor chip CM5 by amine coupling according to the standard procedure until 2300 resonance units (RU) were coupled (1000 RU corresponds to approximately 1.0 ng/mm² or 6 mg/mL; see BIAlite manual). Binding of 120 nM SecA, 0.425 mg/mL YEG⁺ IMV, or 0.25 mg/mL urea-treated YEG⁺ IMV was performed at 10 μL/min in buffer HKM (50 mM HEPES− KOH, pH 7.5, 100 mM KCl, 5 mM MgCl₂, and 0.002% surfactant P20) and 1.6 mM ADP or AMP-PNP which saturates both NBSs. The association of SecA was followed for 180 s, and the dissociation in the presence of the same concentration of nucleotide was followed for 200 s. Regeneration of the sensor chip after each analysis cycle was performed by injection of 12 μL of 1 M diethanolamine, pH 9.0, followed by the continuous flow of buffer HKM buffer. A control CM5 sensor chip with the same history of treatment as the CM5-300-1K5 chip, apart from the addition of 300-1K5 during the immobilizing step, was used to determine the background binding. The obtained data were subtracted from the data obtained by the 300-1K5 sensor. All experiments were repeated 4−10 times, and the results were averaged. A second CM5 sensor chip with 2000 Ru immobilized anti-mouse IgG Fcγ (Pharmacia Biotech) was used to capture the mAbs against SecA and subsequently to measure the binding of SecA.

The change in the surface plasmon wave vector, k_{sp}, is determined by the change in the dielectric constant and thickness of the polymer layer of biological molecules as follows:

$$\Delta k^p_{sp} = k_0 \left(\frac{\epsilon^r_m \epsilon_s}{\epsilon^r_m + \epsilon_s}\right)^2 \left(\frac{\epsilon_p - \epsilon^r_m}{\epsilon_s - \epsilon^r_m}\right) \left[\frac{1}{(-\epsilon^r_m \epsilon_s)^{1/2}}\right] \left(\frac{\epsilon_p - \epsilon_s}{\epsilon_p}\right) \frac{2\Pi d_p}{\lambda} \quad (1)$$

where Δk^p_{sp} is the contribution of the polymer layer to the plasmon wave vector, ϵ^r_m is the real part of the dielectric constant of the metal layer, ϵ_s and ϵ_p are the dielectric constants of the solution and the polymer layer, respectively, d_p is the thickness of the polymer layer, and k^0 is the wave vector of light in vacuum (*41*).

Table 1: *Escherichia coli* Strains and Plasmids

strains	genotype	source or reference
JM109	*recA1, endA1, gyrA96, thi⁻, hsdR17, relA1, supE44, λ⁻, Δ(lac-proAB),* [F′, *traD36, proAB, lacI*q *ΔM15]*	46
POP2136	F⁻ *ara D139 Δ(lac)U169 thiA rspL relA flaB* cI*ts857*	47
SF100	F⁻ Δ*(lac)X74 galE thiA rspL (strA) ΔphoA (PvuII) ΔompT*	48
NO2947	Δ*lac[IPOZYA] araD139 Δ[ara-leu]7697 galU galK rpsL recA56 srl::*Tn10 rₖmₖ	K. Linton
MM52	secA51ᵗˢ	49
D10	*rna10, rel*A1, *spo*T1, *met*B1	30

plasmids	description	source or reference
pMKL18	wild-type *secA*	50
pET111	*secA* containing aaᵃ 323−706 in pEX1	this work
pET112	*secA* containing aa 323−558 in pEX1	this work
pET113	*secA* containing aa 323−831 in pEX1	this work
pET135	*secA* containing aa 149−323 in pEX2	this work
pEX1, 2, 3	Ampʳ PR promoter of phage λ and *cro-lacZ* gene fusion with multiple cloning site in all three reading frames	32
pET340	*secY, secE, secG* in pTRC99A	8

ᵃ aa is amino acids.

The complex effective refractive index of the surface plasmon (N_{eff}) depends on the angle of reflection (θ) as

$$N_{eff} = n_p \sin \theta \qquad (2)$$

where n_p is the refractive index of the prism. It should be noted that the dielectric constant is the square root of the refractive index.

All sensograms were fitted by nonlinear regression assuming a A + B1 + B2 ↔ AB1 + AB2 model for the binding of SecA to IgG, using the Bia-evaluation program provided by Pharmacia. Under results, only the association rate constant k_a (s⁻¹ M⁻¹), the dissociation rate constant k_d (s⁻¹), and the dissociation constant K_D (M⁻¹) for the major compound (84%) are shown.

RESULTS

Production and Epitope Mapping of MAbs against SecA. To study the structure and function of the amino- and carboxy-terminal domains of SecA, monoclonal antibodies were developed against intact SecA, and against *cro-β*-galactosidase fusions to amino acid residues 323−706 and 149−323 of SecA. Hybridoma supernatants producing antibodies raised against the three proteins were selected for binding the intact SecA, the respective SecA fragment, but not the *cro-β*-galactosidase fusion protein in an ELISA. Ten epitopes of 14 selected mAbs were further characterized with isolated and purified SecA fragments of known polypeptide sequence (*42*) and with some additional *cro-β*-galactosidase−SecA fusion proteins (Tables 1 and 2). The epitopes of the mAbs were refined to five areas on the polypeptide of SecA (Figure 1). The four mAbs obtained from the immunization with the intact SecA (*e.g.*, hybridoma fusion 300, mAb 1K5 in Table 2) all recognize an epitope confined to a carboxy-terminal area containing amino acid residues 720−790. The mAbs obtained from *cro-β*-galactosidase−SecA(149−323) fusion protein recognize an epitope which is at least partly confined in the amino acid sequence 149−237 (*e.g.*, hybridoma fusion 323, mAb 1K1; Table 2). The mAbs obtained from *cro-β*-galactosidase−SecA(323−706) fusion protein recognize three epitopes that are at least partly located in the amino acid sequence 323−441, 438−441, and 502−558 (*e.g.*, hybridoma fusion 322, mAbs 1K1, 2E9, and 1B10, respectively; Table 2). Four mAbs were taken into production and purified from hybridoma supernatant (322-1K1 and

Table 2: Characterization and Epitope Mapping of the MAbs against SecA

	antigen				
	149−323	323−706	323−706	323−706	1−901
SecA fragment	mAbs				
	323-1K1	322-1K1	322-2E9	322-1B10	300-1K5
323−558	−	+	+	+	−
323−706	−	+	+	+	−
323−831	−	+	+	+	+
149−323	+	−	−	−	−
1−790	+	+	+	+	+
1−720	+	+	+	+	−
1−502	+	+	+	−	−
1−441	+	+	+	−	−
1−237	+	−	−	−	−
267−901	−	+	+	+	+
438−901	−	−	+	+	+
epitopeᵇ	149−237	323−441	438−441	502−558	720−790
IgG subclass	IgG2a	IgG2a	IgG1	IgG1	IgG1

ᵃ Numbers refer to the amino acid sequence of SecA. +, reaction; and −, no reaction on ELISA. ᵇ Since the mAbs seem to bind native SecA preferentially, it cannot be excluded that other amino acids outside the determined epitope area contribute to the mAb binding site.
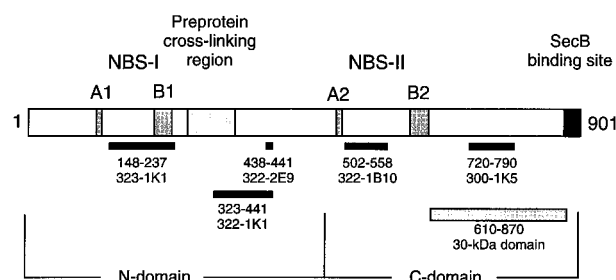

FIGURE 1: Schematic representation of SecA domains, ligand binding sites, epitopes of the mAbs, and the 30-kDa membrane-inserting fragment of SecA (*26*). NBS is nucleotide binding site.

322-2E9) or from ascites (300-1K5 and 323-1K1). Each of these mAbs recognizes SecA as a single band on immunoblots of IMV (shown for 323-1K1, 322-1K1, and 300-1K5 in Figure 2, lanes 3−5, respectively).

ELISA and dot immunoblot were used to access whether any of the mAbs was able to discriminate between denatured and native SecA. SecA was either directly applied to the ELISA or dot immunoblot or first denatured by boiling in Laemmli SDS sample buffer and incubated with the mAbs.
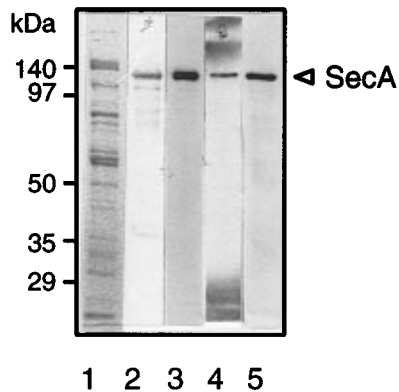
FIGURE 2: Immunolabeling of SecA in vesicles. *E. coli* D10 vesicles (9 μg) were analyzed on a 10% SDS−PAGE and stained with Coomassie brilliant blue (lane 1) or immunoblotted. Immunoblots were developed with a pAb against SecA (lane 2), mAb 323-1K1 (lane 3), mAb 322-1K1 (lane 4), or mAb 300-1K5 (lane 5). On the left, the positions and molecular masses of prestained markers (Bio-Rad) are indicated. MAb 322-1K1 recognizes denatured SecA only poorly, and therefore substantial background staining is visible in lane 4.

Table 3: Native SecA Binding by the MAbs in ELISA and SPR in the IgG Capturing Mode[a]

| | ELISA[b] | | SPR[c] | |
mAb	SecA^N	SecA^D	k_a (s^{-1} M^{-1})	k_d (s^{-1})
300-1K5	100	20	$2.5 \times 10^5 \pm 6 \times 10^3$	$1 \times 10^{-3} \pm 7 \times 10^{-5}$
322-2E9	106	18	$8 \times 10^4 \pm 2 \times 10^3$	$\leq 10^{-4}$
322-1K1	66	6	nd	nd
323-1K1	122	35	$5.6 \times 10^4 \pm 1 \times 10^3$	$2 \times 10^{-3} \pm 8 \times 10^{-5}$

[a] The binding of SecA to the anti mouse IgG Fγ was negligible. [b] A solution of 50 μg/mL SecA was coated on ELISA; the primary antibody concentration was 15 μg/mL. The reaction of mAb 300-1K5 was set at 100%. [c] The dissociation rate is a combination of the release of SecA from the mAb and the release of the mAb from the anti mouse IgG Fcγ. [d] nd is not done.
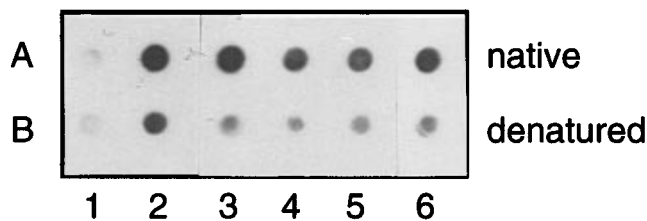

FIGURE 3: Dot immunoblot analysis of the accessibility of the mAb epitopes in native and SDS-denatured SecA. Purified native SecA and SDS-denatured SecA (250 ng/dot) were analyzed on a dot immunoblot with hybridoma supernatant of the mAbs. Lane 1, normal mouse serum (1:1000) in 1% BSA, 0.5% Tween 20 in TBS; lane 2, pAb against SecA (1:20 000) in 1% BSA, 0.5% Tween 20 in TBS; lane 3, mAb 323-1K1 (epitope 149−237); lane 4, mAb 322-1K1 (323−441); lane 5, mAb 322-2E9 (438−441); lane 6, mAb 300-1K5 (720−790). The blot was developed using a chemiluminescence kit (Tropix).

The mAbs against the epitope area, 720−790 (300-1K5), 438−441 (322-2E9), and 149−237 (323-1K1), bind native SecA very well and denatured SecA to a lesser extent (Table 3 and Figure 3). These mAbs were also able to recognize SecA in a surface plasmon resonance biosensor assay, where the purified IgG was bound by anti-mouse IgG Fcγ immobilized on the surface of a sensor chip and the SecA flowing passed the sensor surface (Table 3). SPR detects changes in the refractive index of the surface layer of a solution in contact with the sensor chip (for a review, see ref *41*). The K_D is in the nanomolar range for all mAbs
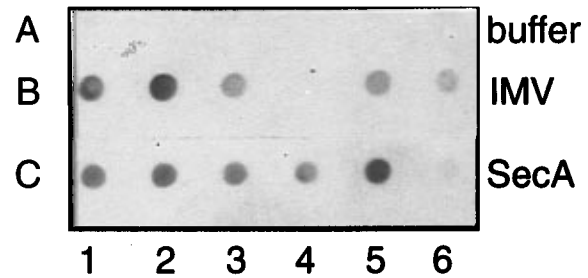

FIGURE 4: Dot immunoblot analysis of the accessibility of the mAb epitopes in IMV of *E. coli* KM9. Buffer, IMV concentration normalized with respect to SecA, and the same amount of purified SecA were analyzed on a dot immunoblot with hybridoma supernatant of the mAbs. Lane 1, mAb 300-1K5 (epitope 720−790); lane 2, mAb 323−2B10 (502−558); lane 3, mAb 322-2E9 (438−441); lane 4, mAb 322-1K1 (323−441); lane 5, mAb 323-1K1 (149−237); lane 6, normal mouse serum (1:1000) in 1% BSA, 0.5% Tween 20 in TBS. The blot was developed using a chemiluminescence kit (Tropix)
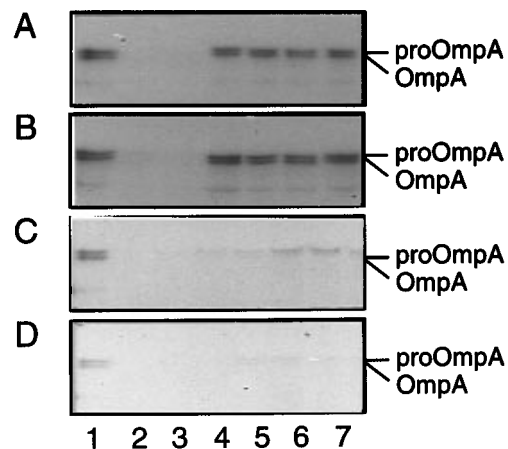

FIGURE 5: Effect of mAbs on the *in vitro* translocation of proOmpA into IMV. Translocation of ^{35}S-proOmpA into urea-treated *E. coli* D10 vesicles and 0.1 μM SecA was assayed in the presence of increasing concentrations IgG (A, C) or Fab (B, D) of mAb 323-1K1 (A, B) and mAb 300-1K5 (C, D). Preprotein translocation in the absence of antibody (lane 1); in the absence of ATP (lane 2); in the absence of SecA (lane 3); and in the presence of 0.1, 0.2, 0.4, and 0.8 μM antibody (lanes 4 up to 7, respectively).

tested. However, the association rates of mAb 323-1K1 and 322-2E9 are slower than that of 300-1K5, which is partly compensated by a decreased dissociation rate compared to 300-1K5. It can be concluded that the mAbs recognize native SecA and to a lesser extent denatured SecA.

A dot immunoblot was used to access whether any of the mAbs was able to recognize the membrane-bound SecA. IMVs were directly applied to the dot immunoblot and incubated with the mAbs. All mAbs apart from mAb 322-1K1 (323−441) were able to bind SecA in this assay (Figure 4), indicating either that their epitopes are accessible in the membrane-associated form of SecA or that the vesicles were sufficiently disrupted to expose their epitopes.

In Vitro Preprotein Translocation Is Inhibited by MAb 300-1K5. A series of mAb dilutions was used to assay their effect on the *in vitro* translocation of proOmpA in wild-type *E. coli* D10 and YEG$^+$ vesicles bearing endogenous SecA (not shown) and in urea-treated vesicles supplied with SecA (Figure 5). At a 2-fold molar excess of mAb to SecA, mAb 300-1K5 inhibited the proOmpA translocation completely irrespective of whether the vesicles were treated with urea. To exclude that inhibition is caused by cross-linking of the SecA dimer (*17*) by the bivalent IgG molecules, Fab
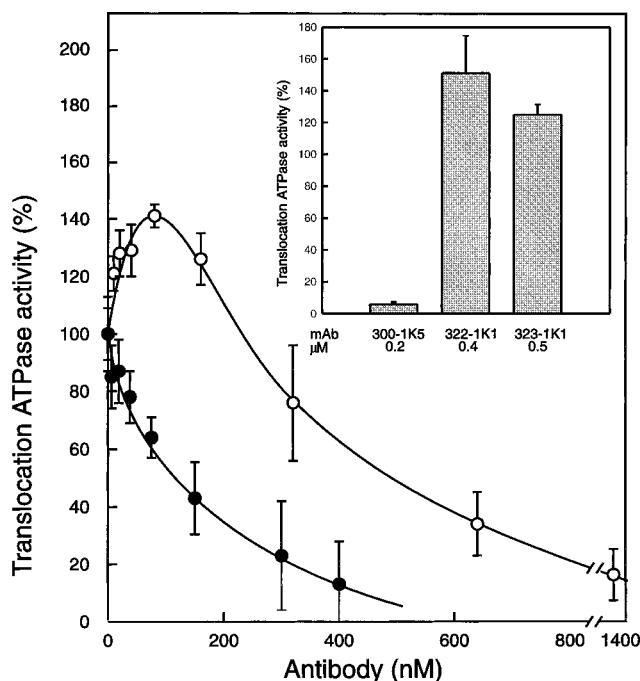
FIGURE 6: Effect of the mAb on the SecA translocation ATPase activity. The activity is expressed as a percentage of the translocation ATPase activity of SecA in YEG+ IMV in the absence of mAb. MAb 300-1K5 IgG (●) and Fab (○) (*n* = 2). The inset shows the effect of mAb IgG 300-1K5, 322-1K1, and 323-1K1 on the translocation ATPase activity of SecA in wild-type IMV. The effect of mAb 322-1K1 and 323-1K1 was assayed at a higher concentration to compensate for the lower SecA binding affinity. The activity is expressed as a percentage of the translocation ATPase activity of SecA in the absence of mAb (*n* = 4). The SecA concentration was 0.085 μM for both experiments.

fragments were prepared by papain digestion. The Fab fragments of mAb 300-1K5 also inhibited proOmpA translocation (Figure 5D). The other mAbs and their corresponding Fabs showed only minor effects on the *in vitro* translocation of proOmpA (shown for mAb 323-1K1 in Figure 5A,B). It is concluded that the mAb against the C-domain inhibits the preprotein translocation reaction, and this is not caused by cross-linking of the SecA dimer.

Translocation ATPase Activity of SecA but Not Its Endogenous ATPase Activity Is Inhibited by MAb 300-1K5. The translocation ATPase activity of SecA was assayed in the presence of increasing concentrations of mAb, using proOmpA as substrate. At a 3-fold excess, mAb 300-1K5 IgG inhibited the translocation ATPase activity completely (Figure 6), whereas the Fab stimulated the activity when present at substoichiometric amounts but inhibited the reaction completely at a 10-fold excess relative to SecA (Figure 6). The antibody had no effect on the endogenous ATPase activity (results not shown). The other mAbs show a slight stimulation of the translocation (Figure 6, inset) and endogenous ATPase activity of SecA (results not shown). Summarizing, the mAb against the C-domain inhibits the translocation but not the endogenous ATPase activity of SecA.

MAb 300-1K5 Interferes with SecA Membrane Insertion and De-insertion. SecA inserts into the membrane in the presence of ATP and preprotein or in the presence of a nonhydrolyzable ATP analogue such as AMP-PNP (24, 14). Membrane insertion is assayed as the formation of a 125I-labeled 30-kDa SecA domain that is inaccessible to proteases (24). We used this assay, both under translocation (ATP)
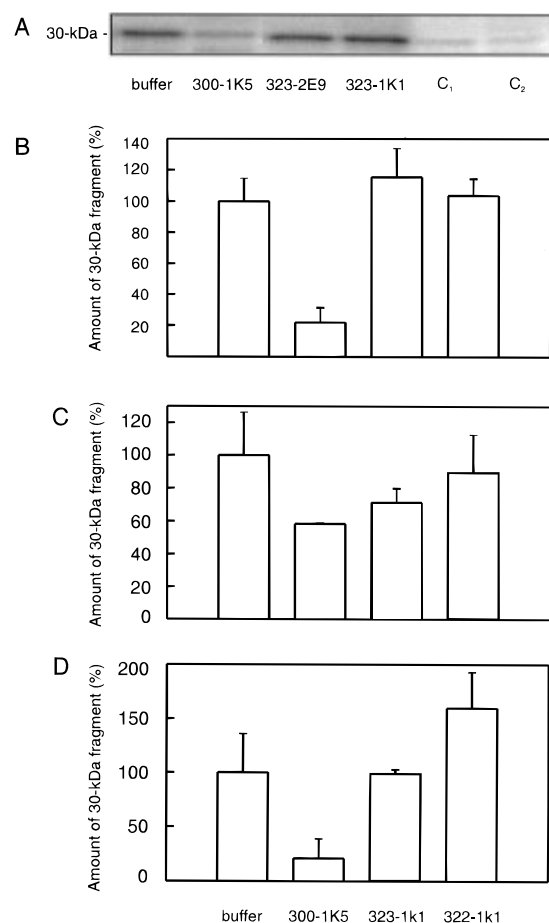

FIGURE 7: SecA membrane insertion is inhibited by mAb 300-1K5, but not by the other mAbs. Protection of a 125I-labeled 30-kDa fragment of SecA against proteolytic digestion was used as a measure for the insertion of 125I-SecA (16 nM) in urea treated *E. coli* SecYEG+ vesicles in translocation buffer containing SecB and proOmpA. Incubation at 0 °C with 1 mM AMP-PNP (A, B), incubation at 37 °C with 1 mM ATP (C), and incubation at 37 °C with 1 mM AMP-PNP (D). Samples were incubated for 30 min in the presence or absence of mAb IgG (48 nM). The formation of the 30-kDa fragment is expressed as a percentage of the control that was incubated in the absence of mAbs (*n* = 2). C_1 and C_2 in panel A show the protection of the 30-kDa fragment in the absence of nucleotide and vesicles, respectively.

and under non-translocation (AMP-PNP) conditions, to analyze the influence of the mAbs on the membrane insertion and subsequent de-insertion of SecA. MAb 300-1K5 inhibits the insertion of SecA under both conditions (Figure 7), albeit the effect is somewhat weaker under translocation conditions (Figure 7C). The other mAbs showed no inhibition of the insertion of SecA (Figure 7). To substantiate the inhibition of membrane insertion by mAb 300-1K5, we followed the formation of the 30-kDa SecA fragment in the absence and presence of this mAb by titration with increasing concentrations of AMP-PNP or ADP. ADP did not induce the protection of a 30-kDa fragment (results not shown). The protection of the 30-kDa fragment is completely abolished by mAb 300-1K5 (Figure 8, closed circles), indicating that the mAb interferes with the membrane insertion of SecA. Trp fluorescence studies demonstrate that NBS-I binds AMP-PNP with similar affinity as ADP (18). The induction of the protection of the 30-kDa fragment occurs at a much higher AMP-PNP concentration (open circles) than needed to occupy NBS-I only. This implies that both NBS-I and NBS-II have to be occupied by AMP-PNP.
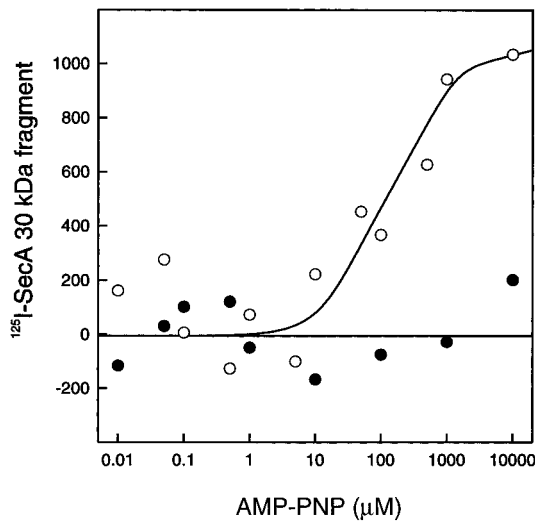
FIGURE 8: AMP-PNP-induced formation of the protected 30-kDa fragment is inhibited by mAb 300-1K5. The amount of ^{125}I-labeled 30-kDa SecA fragment as a function of the AMP-PNP concentration in the absence (○) or presence (●) of mAb 300-1K5. After 10 min of preincubation at 37 °C, AMP-PNP (0.01−10 000 μM) was added to urea-treated *E. coli* YEG$^+$ vesicles in translocation buffer containing SecB, proOmpA, and 16 nM ^{125}I-SecA and as indicated 48 nM 300-1K5 IgG. Reactions were further incubated for 20 min at 37 °C ($n = 2$), and subsequently digested with proteinase K (1 mg/mL) and analyzed by Phosphorimaging as described.

Next, the effect of the mAbs on the de-insertion reaction of SecA was addressed. ^{125}I-SecA was allowed to insert into the membrane in the presence of AMP-PNP for 20 min. Subsequently, the mAbs were added, and the incubation of the sample was continued. Alternatively, an excess of unlabeled SecA which competes with the ^{125}I-SecA for insertion (*24*) was added. Within 5 min of the addition of a 4-fold excess of mAb 300-1K5 IgG, about 55% of the labeled SecA was de-inserted, whereas addition of a 12-fold excess of unlabeled SecA de-inserted only 30% of the inserted ^{125}I-SecA (Figure 9). MAbs 322-1K1 and 323-1K1 had a similar effect as buffer on the de-insertion of SecA. A 12.5-fold excess of 300-1K5 Fab also stimulated the de-insertion of SecA, although a 2.5-fold excess stimulated the insertion reaction (results not shown). This corresponds to the effect of the 300-1K5 Fab on the translocation ATPase activity of SecA (Figure 6). The reversal of the insertion by mAb 300-1K5 in the presence of ATP was again somewhat slower than in the presence of AMP-PNP (results not shown). Since the mAb 300-1K5 more rapidly and more efficiently affects the membrane de-insertion of ^{125}I-labeled SecA as compared to the replacement reaction in the presence of an excess of unlabeled SecA, it is concluded that the mAb actively reverses the membrane insertion of SecA.

MAb 300-1K5 Specifically Stimulates Membrane De-insertion of SecA. To check whether the stimulation of the de-insertion of the 30-kDa fragment occurs concomitantly with the dissociation of SecA from the membrane, the effect of the mAbs on the release of SecA from the membrane was studied. YEG$^+$ vesicles were incubated with ^{125}I-SecA in the presence of ATP or AMP-PNP and subsequently centrifuged through a sucrose cushion to remove unbound material. Vesicles were resuspended in the presence of unlabeled SecA or mAbs and incubated for 10 min at 37 °C. The dissociated SecA was separated from the vesicles by centrifugation through a sucrose cushion. In the presence of AMP-PNP and unlabeled SecA, mAb 300-1K5 IgG or
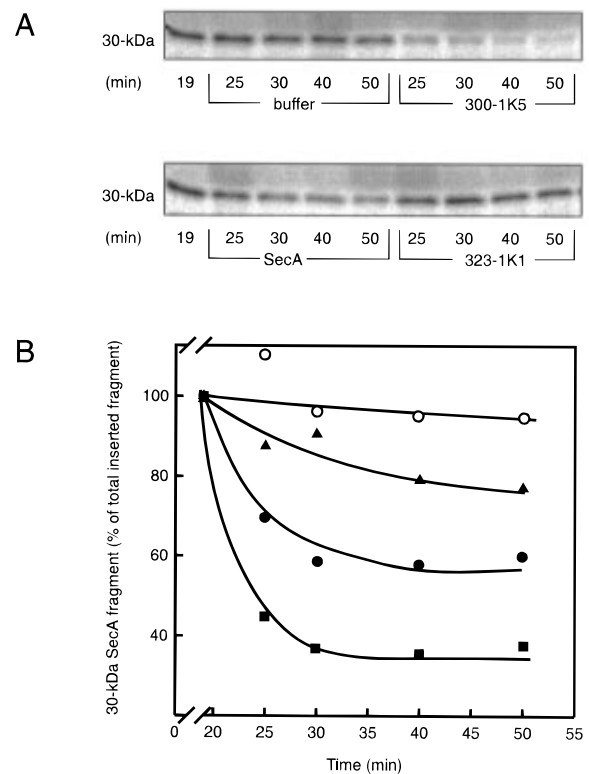

FIGURE 9: SecA membrane de-insertion is stimulated by mAb 300-1K5. Protection of a 30-kDa fragment of ^{125}I-SecA against proteolytic digestion was used as a measure for the de-insertion of ^{125}I-SecA (16 nM) in urea-treated *E. coli* SecYEG$^+$ vesicles in translocation buffer containing SecB and proOmpA. Samples were incubated at 37 °C in the presence of 1 mM AMP-PNP for 19 min (100%), and incubation proceeded for the indicated times in the presence of buffer (○) or a 12-fold molar excess of unlabeled SecA (●) or a 4-fold excess of mAb 300-1K5 (■) or 323-1K1 IgG (▲). The amount of 30-kDa fragment was visualized by Phosphorimaging (A) and quantitated as a percent of the amount present after 19 min incubation in the presence of AMP-PNP (B).

Fab released 45% of the SecA from the membrane, whereas mAbs 322-1K1 or 323-1K1 had similar effects as incubation in buffer (Figure 10A). This shows that mAb 300-1K5 not only stimulates SecA membrane-deinsertion but also promotes the release of SecA from the membrane. MAb 300-1K5 IgG in the presence of ATP did not dissociate SecA from the membranes in the time course of the experiment whereas 300-1K5 Fab did (Figure 10B). In view of the overall slower effects on SecA insertion and de-insertion of this mAb in the presence of ATP as compared to AMP-PNP, this result suggests that the epitope for mAb 300-1K5 is more accessible in the AMP-PNP than in the ATP inserted state of SecA.

Surface Plasmon Resonance Detection of Nucleotide-Induced Conformational Changes of Soluble and Membrane Inserted SecA. The ADP- and AMP-PNP-bound states of SecA differ in conformation and compactness (*18*). The AMP-PNP-bound state is more extended than the ADP-bound state. To investigate whether mAb 300-1K5 has a preference for either of these conformations of SecA, the mAb was immobilized on a sensor. The binding of SecA or YEG$^+$ IMV to the 300-1K5-sensor was followed as a function of resonance units (RU) using the SPR technique. The variation in the experiments ($n = 4−10$) was less than 1% for the ADP-bound conformations, and 7% and less than 1% for the AMP-PNP-bound conformation of soluble SecA and SecA in vesicles, respectively. The decrease in repro-
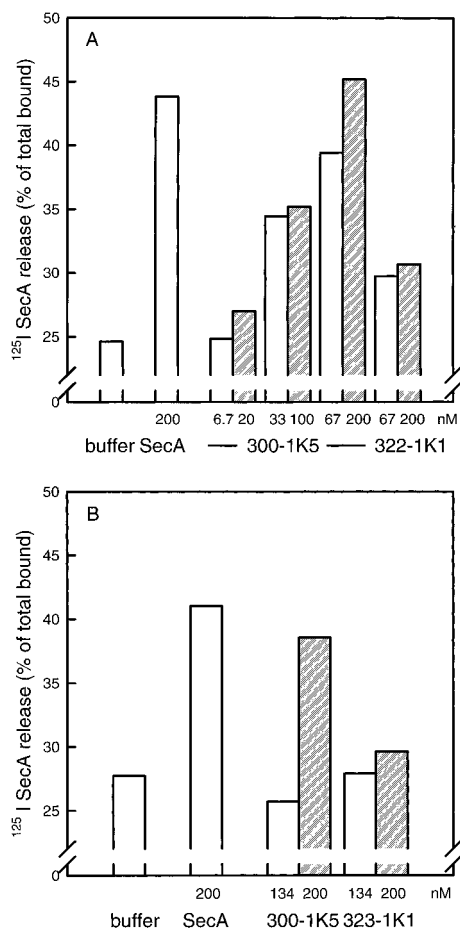
FIGURE 10: Release of SecA from the membrane is stimulated by mAb 300-1K5 IgG (white bars) and Fab (gray bars). Urea-treated *E. coli* SecYEG$^+$ vesicles were first incubated with ^{125}I-labeled SecA (16 nM), and the vesicles with bound SecA were isolated by centrifugation through a sucrose cushion. The vesicles were subsequently diluted in buffer with AMP-PNP (A) or with ATP (B) at 37 °C in the absence and presence of SecA, mAb 300-1K5, or mAb 322-1K1. After 10 min incubation, the amount of membrane-associated and released SecA was determined after separation of the samples in a pellet and supernatant fraction by centrifugation through a sucrose cushion. The amount of released SecA is expressed as the percentage of total membrane bound SecA.

ducibility in the presence of AMP-PNP in the absence of vesicles is probably caused by aggregation of SecA. At identical SecA concentrations applied, the number of bound resonance units is 25% higher for the AMP-PNP-bound SecA than for the ADP-bound SecA (Figure 11A). The association rate is the same for both SecA conformations, while the dissociation rates differ by a factor 1.5 (Table 4). This does not account for the 25% difference in resonance units. SPR is measured as the change in surface plasmon angle (θ) at which resonance occurs. Resonance occurs if the plasmon wave vector (k_{sp}, eq 1) is equal to the light wave vector of the incident light. The surface plasmon wave vector is proportional to the thickness of the layer containing the immobilized ligand and bound analyte, and to the dielectric constant or to the refractive index of the analyte (*41*). Changes on the surface of the sensor, will change the surface plasmon wave vector and therefore the angle at which resonance occurs (eq 2). Because most biological polymers have a refractive index between 1.5 and 1.6 (*43*), the maximum change in refractive index would be 6.7%. This would cause a change in the surface plasmon angle of about 16% (eq 2). The change in resonance signal is therefore more likely an indication that the AMP-PNP-bound SecA
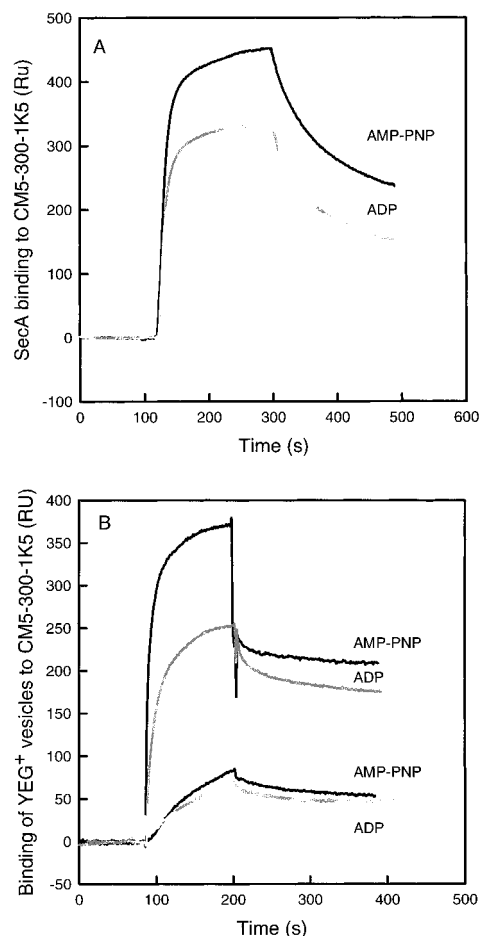

FIGURE 11: Surface plasmon resonance sensogram of the binding of 120 nM SecA (A) or 0.425 mg/mL YEG$^+$ IMV (upper sensograms in panel B) or 0.25 mg/mL urea-treated YEG$^+$ IMV (lower sensograms in panel B) in the presence of 1.6 mM AMP-PNP (black lines) or ADP (gray lines). The curves illustrate the association and dissociation as a function of time. The concentration of SecA present in the samples containing YEG$^+$ vesicles was 90 nM. The sensograms of aspecific binding of SecA or YEG$^+$ IMV to the sensor dextran layer were subtracted. The sensograms are the average of 4−10 experiments.

has a significantly larger hydrated volume than the ADP-bound SecA, thereby changing the refractive index as well as the thickness of the layer. Similar changes in hydrated volume have been observed for *B. subtilis* SecA (*18*).

SecA associates with IMV in the presence of ADP, but does not insert in the membrane to the extent that the 30-kDa fragment is protected against proteinase K digestion, whereas it does in the presence of AMP-PNP (*24*). To access whether the membrane-bound SecA has a nucleotide-dependent conformation similar to SecA in solution, YEG$^+$ IMV containing intrinsically bound SecA in the presence of ADP or AMP-PNP were injected in the 300-1K5 sensor compartment, and the association was followed for 120 s as described above. The association rate of AMP-PNP YEG$^+$ vesicles is twice as fast as that of ADP YEG$^+$ vesicles, whereas the dissociation rates are the same (Table 4). This is primarily due to the combined binding of SecA and vesicles as a consequence of the AMP-PNP-induced membrane inserted state of SecA, adding more mass. In the presence of ADP, the SecA seems to dissociate rapidly from the vesicles during the association with 300-1K5 (Figure 11B). The dissociation phase of the SecA inserted in the vesicles in the presence of AMP-PNP is again a combined effect of a very fast dissociation of the vesicles from SecA

Table 4: Kinetic Association and Dissociation Rates of the SecA and YEG$^+$ IMV Interaction with 300-1K5 IgG Immobilized on a CM5-Sensor

sample	k_a (s^{-1} M^{-1})	k_d (s^{-1})	K_D (nM)
SecA AMP-PNP	$1.22 \times 10^5 \pm 2.56 \times 10^4$	$2.95 \times 10^{-3} \pm 1.52 \times 10^{-4}$	24
SecA ADP	$1.11 \times 10^5 \pm 7.93 \times 10^3$	$4.49 \times 10^{-3} \pm 1.39 \times 10^{-4}$	40
YEG$^+$ AMP-PNP	$2.14 \times 10^5 \pm 2.37 \times 10^4$	$9.0 \times 10^{-4} \pm 3.12 \times 10^{-4}$	4.2
YEG$^+$ ADP	$1.12 \times 10^5 \pm 2.52 \times 10^4$	$1.08 \times 10^{-3} \pm 5.37 \times 10^{-5}$	9.6

and a much slower dissociation of SecA from the mAb. The epitope located between amino acid residues 720−790 of 300-1K5 is apparently at least partly accessible in the membrane inserted state of SecA. No dissociation phase of the ADP YEG$^+$ vesicles is observed because the vesicles did not remain bound to SecA during the association phase. After a dissociation period of 200 s, the resonance signal is still 22% higher for AMP-PNP-bound SecA than for ADP-bound SecA, indicating similar conformations as described above.

Treatment of IMV with urea removes approximately 70% of the membrane-associated proteins (*9*), but leaves some of the SecA membrane-bound in a translocation competent form (*8*). The immobilized 300-1K5 binds only minor amounts of the urea-treated YEG$^+$ vesicles (lower traces in Figure 11B). Normalizing the protein content of the vesicles to the YEG complex present in the urea-treated and non-treated vesicles, the amount of specifically bound SecA in the urea-treated vesicles is about 13% of the amount bound in the nontreated vesicles. These data indicate that mAb 300-1K5 does not discriminate between the ADP- and AMP-PNP bound conformational states of SecA, and that the epitope present in the C-domain of SecA is accessible to the mAb both in the membrane-surface associated and inserted states.

DISCUSSION

In this paper, we describe the production of monoclonal antibodies against five different epitopes of SecA and their effect on SecA preprotein translocation. All mAbs recognized purified native SecA and, to a lower extent, denatured SecA. The mAbs, apart from 322-1K1, recognize IMV-bound SecA on a dot immunoblot. In the case of the membrane associated/inserted SecA, this determination is more complex since SecA can easily dissociate from the vesicles during the incubation procedure, for instance due to disruption of the membranes while they bind to the supporting material. No reliable indication could be obtained that mAb 323-1K1 or mAb 322-2E9 was able to bind the membrane inserted SecA. As measured by surface plasmon resonance, the SecA association rate of these mAb was on the order of 10^4 s^{-1} M^{-1}, and may be too slow to effectively compete with the membrane association of SecA. In the absence of ATP, SecA already has a K_D of $\approx$40 nM for vesicles (*3*). Alternatively, binding of the mAb to the epitopes may have little effect on the activity of SecA.

Inhibition of the Translocation of proOmpA by MAb 300-1K5. The mAb 300-1K5 inhibits the translocation of proOmpA and the translocation ATPase activity of SecA, but not its endogenous ATPase activity. It recognizes an epitope which includes at least several amino acids between positions 720 and 790. SecA mutants which have lost more than the last 70 carboxy-terminal amino acids are not able to translocate preproteins *in vivo* (*38*), and mutants lacking the last 66 amino acids are severely impaired in protein

translocation (*44*). A carboxy-terminal deletion up to amino acid residue 720 does impair the translocation but not the endogenous ATPase activity (*38*). The carboxyl terminus is apparently not required for the ATPase activity of SecA, but rather for the coupling of ATP hydrolysis to preprotein translocation. As demonstrated by the surface plasmon resonance experiments, mAb 300-1K5 is able to bind and recognize both the ADP bound and the more extended AMP-PNP bound conformation of SecA, suggesting that nucleotide binding is not seriously impaired by the mAb. The carboxyl terminus has also been reported to be involved in the binding of SecB (*45*; P. Fekkes, unpublished results). The inhibition of preprotein translocation by mAb 300-1K5 is not dependent on the presence of SecB (results not shown), implying that SecB is clearly not involved.

Accessibility of the Epitope of MAb 300-1K5. The inhibition of the proOmpA translocation is a consequence of the inability of SecA to insert into the cytoplasmic membrane in the presence of the antibody. The mAb also stimulated the de-insertion and the dissociation of SecA from the membrane. It has been proposed that SecA contains at least two membrane associating domains, possibly located between amino acid residues 64 and 239 and amino acids 831−901 (*9, 38, 42*). The epitope of mAb 300-1K5 does not fall within this range, but is part of the 30-kDa carboxy-terminal fragment (amino acids 610 to approximately 870) that has been shown to insert upon ATP binding (*26*). This region just precedes the proteolytic site in the vicinity of amino acid residue 850 of SecA that has been shown to be accessible from the periplasmic side of the membrane (*8*), consistent with the notion that part of the C-domain of SecA inserts into the membrane (*26*). Since mAb 300-1K5 is able to bind the membrane inserted state of SecA, it can be concluded that its epitope is not concealed by the membrane or by the integral membrane subunits of the *translocase* during at least part of the preprotein translocation process.

The distribution between the membrane and cytosolic states of SecA is likely a dynamic process of which the equilibrium is affected by nucleotides (*24, 25*). ADP directs the protein to the surface-bound state or cytosolic state, whereas ATP favors the membrane inserted state. Release of SecA from inner membranes can be induced by the addition of an excess of unlabeled SecA (*24, 14*). This process is dependent on ATP hydrolysis (*24*) but not on preprotein translocation (*14*). The amount of SecA that can be released from these membranes is dependent on the method of SecA depletion, i.e., urea treatment (*24*) or depletion of the SecA during growth of a *secA* temperature-sensitive strain (*14*). Urea removes about 70% of the proteins associated with the membrane (*9*). It is feasible that this treatment removes or inactivates a component needed for the stable insertion of SecA. We used urea treated membranes reconstituted with ^{125}I-SecA to monitor the de-insertion and the release of SecA from the membrane. The mAb 300-1K5 stimulated both processes far more efficiently

than an excess of SecA, indicating that the mAb actively reverses the inserted state of SecA. The mAb also inhibits the translocation of proOmpA by native IMV which contain endogenous SecA, indicating that the inhibitory effect of the mAb is not specific for the pretreatment of the membranes.

If the mAb would prevent the re-insertion of SecA in the membrane as part of a dynamic equilibrium, it would be expected that the reversion of the insertion of SecA is more pronounced in the ATP-bound state than in the AMP-PNP-bound state. Since the opposite phenomenon is observed, it can be concluded that the mAb recognizes the membrane inserted state of SecA, but that the accessibility of its epitope differs between the ATP and AMP-PNP inserted states of SecA.

The mAb inhibits insertion and stimulates de-insertion irrespectively whether proOmpA translocation occurs (ATP) or not (AMP-PNP). The AMP-PNP-induced insertion of SecA does not require the presence of preprotein (*25*), and therefore it also seems unlikely that mAb 300-1K5 acts by interference with the binding of the preprotein. It seems more likely that the mAb inhibits and reverses a conformation of SecA which is essential for membrane insertion and which differs from the thus far described ADP- or AMP-PNP-bound soluble conformations.

In conclusion, we have isolated a mAb that recognizes an epitope that is located in the carboxy-terminal 30-kDa membrane inserting fragment of SecA. This mAb efficiently inhibits preprotein translocation by interfering with the membrane inserted state of SecA. These data provide further evidence that preprotein translocation and membrane insertion of the C-domain of SecA are coupled processes.

ACKNOWLEDGMENT

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