SecY–SecY and SecY–SecG contacts revealed by site-specific crosslinking

Eli O. van der Sluis, Nico Nouwen, Arnold J.M. Driessen*

Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

Abstract **Protein translocation across the cytoplasmic membrane of *Escherichia coli* is mediated by the integral membrane complex SecYEG and the peripherally bound ATPase SecA. To probe the environment of the cytoplasmic domains of SecY within the SecYEG complex, we introduced single cysteine residues in each of the six cytoplasmic domains. Neighbouring SecY molecules with a single cysteine residue in cytoplasmic domains C1, C2 or C6 formed a disulfide bond upon oxidation. The presence of the disulfide bond between two C2 domains reversibly inhibited protein translocation. Chemical crosslinking showed that the C2 and C3 domains are in close proximity of SecG and chemical modification of the cysteine residue in the C5 domain with *N*-ethyl-maleimide or fluorescein-5-maleimide inactivates the SecYEG complex. Taken together, our data give novel insights in the interactions between subunits of the SecYEG complex and emphasise the importance of cytoplasmic domain C5 for SecY functioning. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

Key words: Protein translocation; SecY; SecG; Cysteine labeling; Crosslinking

1. Introduction

The heterotrimeric membrane protein complex SecYEG forms the protein conducting channel through which precursor proteins are translocated across the prokaryotic cytoplasmic membrane. Together with the peripheral motor protein SecA, SecYEG constitutes a multisubunit complex termed 'translocase'. SecA binds to the SecYEG complex and powers the translocation process by undergoing multiple conformational changes upon binding and hydrolysis of ATP (for a recent review see [1]).

Electron microscopy studies have revealed that multiple SecYE(G) protomers assemble into an oligomeric complex with a pore like structure that resembles the eukaryotic Sec61αβγ complex [2–6]. Biochemical evidence for an oligomeric assembly of SecYEG comes from disulfide crosslinking [7,8], analytical ultracentrifugation, 2D crystallography [9] and Blue-native gel electrophoresis studies (BN-PAGE) [10]. Oligomerisation of SecYEG was shown to be triggered by SecA [4] but the exact oligomeric state of SecYEG during translocation remains a topic of debate [4,10,11].

Over the years, genetic and biochemical experiments have given a substantial insight in the interactions between the different subunits (for a recent review see [12]). In short, SecE interacts with domains of SecY located in the cytoplasm [13,14], the transmembrane region [7,8], and the periplasm [15,16]. Furthermore, SecE is involved in the association of two SecYEG protomers [8]. Disulfide crosslinking of SecE to a neighbouring SecE or SecY molecule inactivates SecYEG, indicating that dynamics of SecE are required during protein translocation [7,8,16].

A direct interaction between SecY and SecA has been demonstrated by aspecific crosslinking studies [17], but so far no site-specific crosslinks have been reported. Genetic studies with cold-sensitive SecY mutants have indicated that the two carboxy-terminal cytoplasmic domains of SecY are involved in an interaction with SecA [18–20]. On the basis of studies with SDS-denatured SecY [21] and peptide antibodies [22] it has been proposed that SecA also interacts with the extreme amino-terminal region of SecY.

SecG is an auxiliary translocase component that strongly facilitates translocation [23–25] and has been shown to be involved in membrane cycling of SecA [26,27]. SecG forms a stable complex with SecYE [28] that can be purified via affinity tags on both SecY and SecE [29,30]. Within the SecYEG complex, SecG can form homodimers that remain associated and even undergo topology inversion during protein translocation [31,32], but the position of SecG with respect to SecY and SecE within the SecYEG complex has remained obscure. Likewise, the domains of SecY involved in the association of SecYEG protomers have never been determined.

In order to understand the molecular mechanism of protein translocation, more detailed structural information on the interactions between the individual subunits within the SecA/SecYEG complex is required. In this study, we have investigated the cytoplasmic side of SecY by introducing single cysteine residues and performing cysteine-specific labeling and crosslinking experiments. The results are discussed with respect to the interactions between the individual subunits and the oligomeric assembly of SecYEG.

2. Materials and methods

2.1. Chemicals and biochemicals

M-Maleimidobenzoyl-*N*-hydroxysuccinimide ester (S-MBS) was purchased from Pierce (Rockford, IL, USA), dithiothreitol (DTT) from Roche (Basel, Switzerland), fluorescein-5-maleimide (F-Mal) and Alexa-fluor® 633 C5-maleimide (AF633) from Molecular Probes (Eugene, OR, USA). All other chemicals were purchased from Sigma (St. Louis, MO, USA). SecA [33], SecB [34] and proOmpA [35] were purified essentially as described. Antibodies against the C-terminus of

*Corresponding author. Fax: (31)-50-3632154.
E-mail address:* a.j.m.driessen@biol.rug.nl (A.J.M. Driessen).

SecG were a kind gift from Hajime Tokuda (University of Tokyo, Tokyo, Japan).

2.2. Bacterial strains, growth conditions and isolation of inner membrane vesicles (IMVs)

Escherichia coli strain DH5-α was used for standard DNA manipulations. Expression of SecYEG was performed in E. coli SF100. IMVs were isolated as described previously [7] except that all steps were performed in 50 mM HEPES/NaOH pH 7.0, 2 mM DTT. Membranes were stored at a protein concentration of 10 mg/ml in the same buffer supplemented with 20% (v/v) glycerol.

2.3. Plasmids

Vector pEK1 containing the cysteine-less secY gene was created by ligating a NcoI–ClaI fragment from pET607 [7] into pET401, resulting in pEK1. pET401 is a pBluescript SK+ (Stratagene, La Jolla, CA, USA) derivative, from which the PstI, SmaI and ApaI sites were removed and a NcoI site was introduced (K.H.M. van Wely, unpublished results). SecY point mutations were introduced with the Stratagene QuikChange™ mutagenesis kit using pEK1 as template. Mutant secY genes were recombined with (cysteine-less) secE and secG genes by exchanging a NcoI–ClaI fragment with pET607, resulting in the plasmids listed in Table 1. All mutations were confirmed by sequence analysis.

2.4. Labeling of cysteines

Before each labeling or crosslinking experiment, IMVs were incubated with 5 mM DTT (30 min, 37°C). Subsequently, IMVs were diluted with 1 ml de-aerated ice-cold 50 mM HEPES/NaOH pH 7.0 and pelleted in a TLA100.2 rotor (20 min, 90 000 rpm). Labeling of the cysteines was performed immediately after resuspension of the membrane pellets in 50 mM HEPES/NaOH pH 7.0 with either 1 mM NEM or 0.25 mM F-Mal for 15 min at 20°C. Labeling reactions were quenched by addition of a 10-fold excess of DTT and membranes were re-isolated as described above. F-Mal-labeled inner membrane proteins were visualised by in gel UV fluorescence on a Roche Lumi-Imager F1 using a cut-off filter of 520 nm.

2.5. Crosslinking

Before crosslinking, IMVs were treated with DTT and re-isolated as described. Oxidative crosslinking was performed with 1 mM tetrathionate (S_4O_6) (30 min, 37°C). Cleavage of disulfide bonds (re-reduction) was performed identically to the reduction procedure described. Chemical crosslinking was performed with 1 mM S-MBS (15 min, 20°C). Reactions were quenched with 100 mM Tris–HCl pH 8.0, 10 mM DTT.

2.6. Translocation assays

In vitro translocation reactions were performed essentially as described [36]. Briefly, 2 μg of IMVs was incubated at 37°C in buffer A (50 mM HEPES/NaOH pH 7.0, 20 mM NaCl, 5 mM MgCl$_2$, 0.1 mg/ ml BSA, 10 mM DTT) with fluorescent proOmpA (1.25 μg/ml), SecA (10 μg/ml), SecB (35 μg/ml), 0.5 mM ATP, 10 mM phosphocreatine and 50 μg/ml creatine kinase. F-Mal or AF633-labeled proOmpA(C302S) was used as the substrate (J. de Keyzer et al., manuscript in preparation). DTT was omitted from buffer A in translocation reactions with oxidised membranes. After 7 min at 37°C, translocation reactions were terminated by chilling on ice and untranslocated proOmpA was digested by proteinase K (0.1 mg/ml, 15 min, 4°C). Protease-protected material was precipitated with 5%

TCA, washed with ice-cold acetone and separated by 12% SDS–PAGE. Fluorescent proOmpA was visualised by in gel UV fluorescence on a Roche Lumi-Imager F1 using a cut-off filter of 520 nm (F-Mal-labeled proOmpA) or 645 nm (AF633-labeled proOmpA).

2.7. Miscellaneous

SDS–PAGE and Western blotting were performed according to standard protocols. Protein concentrations were determined with the Bio-Rad RC DC protein assay kit using BSA as a standard. ProOmpA-stimulated SecA ATPase assays were performed as described [7].

3. Results

3.1. Construction and chemical modification of single cysteine residues in SecY

To probe for intermolecular interactions at the cytoplasmic side of SecYEG by thiol chemistry, we introduced single cysteine residues in each of the six cytoplasmic domains of SecY (C1 to C6, see Fig. 1). To ensure accessibility of the introduced cysteine residues the positions were selected in the middle of each domain. In the selection process, highly conserved residues or positions harbouring cold-sensitive or prl mutations were avoided and where possible serine residues were replaced. In this way, six single cysteine SecY mutants (Table 1) were constructed that were introduced into a cysteine-less secYEG expression system [7].

Inverted membrane vesicles (IMVs) were isolated from E. coli SF100 cells that overproduced the mutant SecYEG complexes. For all cysteine mutants the expression levels of SecYEG and the amount of SecA associated with the membranes were similar to that of the Cys-less control. Furthermore, the proOmpA-stimulated SecA ATPase activity (data not shown) and the in vitro translocation of proOmpA (Fig. 3A) were indistinguishable from the cysteine-less control. We have previously shown that due to the high expression levels achieved with our expression system, the background activity of chromosomally encoded SecYEG is negligible as compared to the plasmid-encoded SecYEG [7,37]. Taken together, this indicates that the introduced cysteines do not alter the expression nor the functionality of the SecYEG complex.

To check the accessibility of the introduced cysteines for thiol modifying reagents, we incubated IMVs harbouring the mutant SecYEG complexes with the thiol reactive probe F-mal. Except for the Cys-less control, all single cysteine mutants show a prominent fluorescent band (Fig. 2) corresponding to SecY as confirmed by Western blotting (data not shown). Due to the high expression levels of SecY(EG), background labeling of other membrane proteins is negligible. Pretreatment of the membranes with NEM completely prevented F-Mal labeling (data not shown). These results show that all

Table 1
Overview of plasmids used in this study

| Plasmid	Relevant characteristics	SecY mutation	Source
pET401	pBluescript SK+ derivative	–	K.H.M. van Wely (unpublished results)
pET607	cysteine-less secYEG in pET610	–	[7]
pEK1	cysteine-less secY in pET401	–	this work
pEK20	cysteine-less secYEG	–	this work
pEK21	secY(S11C)EG	S11C (AGT→TGT)	this work
pEK22	secY(S111C)EG	S111C (TCT→TGT)	this work
pEK23	secY(T179C)EG	T179C (ACT→TGT)	this work
pEK24	secY(S262C)EG	S262C (AGC→TGC)	this work
pEK25	secY(S349C)EG	S349C (TCC→TGC)	this work
pEK26	secY(S431C)EG	S431C (TCT→TGT)	this work
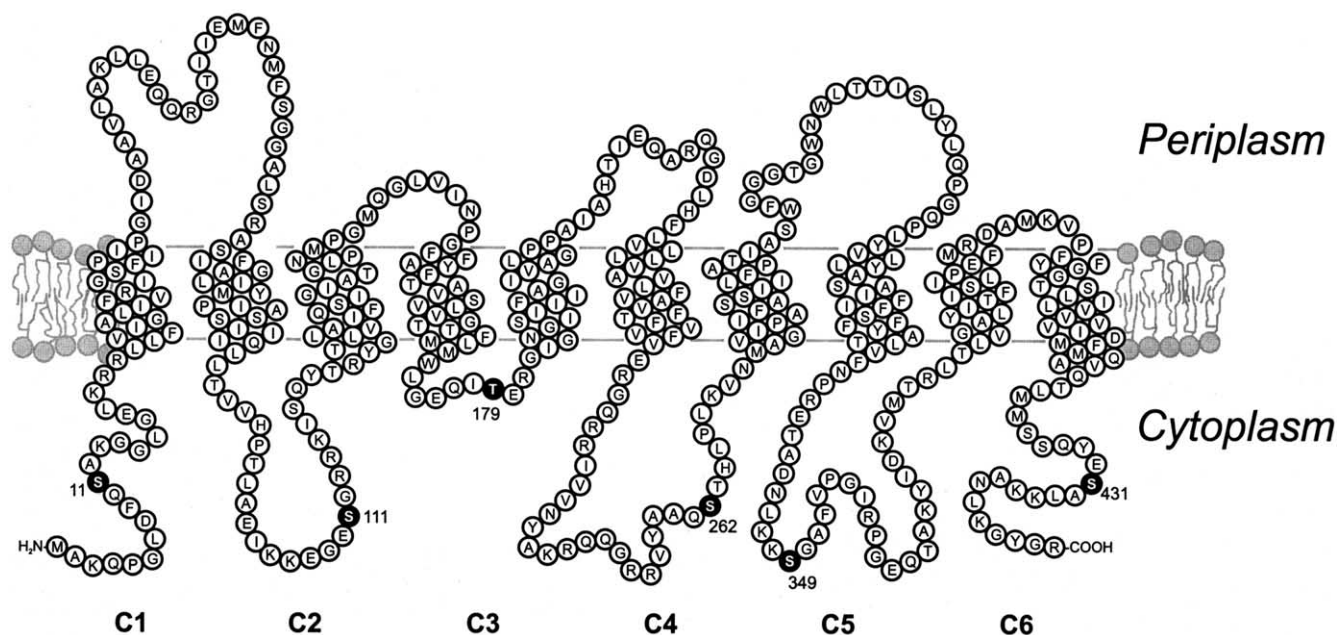
Fig. 1. Membrane topology model of *E. coli* SecY. The residues that were replaced by cysteine are indicated in black circles. The abbreviations used for the cytoplasmic domains (C1 to C6) are indicated below. Endogenous cysteines at positions 329 and 385 have been replaced by a serine residue [7].

the introduced cysteine residues are accessible for the thiol modifying reagents NEM and F-Mal. Furthermore, by using F-Mal as a second round labeling agent, this assay provides a simple tool to assess SecY modification by non-fluorescent thiol reactive probes.

3.2. ProOmpA translocation into IMVs containing chemically modified SecYEG

To investigate if chemical modification of the cytoplasmic cysteine residues affects the functionality of the SecYEG complex, IMVs containing the different SecYEG mutants were treated with NEM (molecular weight (MW) 125 Da) or F-Mal (MW 427 Da). Subsequently, the IMVs were assayed for in vitro translocation activity of proOmpA(C302S) labeled with F-Mal (proOmpA-F-Mal) or with AF633 (proOmpA-AF633). Both these fluorescent precursors behave indistinguishably from unlabeled proOmpA as shown by SecA ATPase activity assays and immunodetection (J. de Keyzer et al., manuscript in preparation) and can thus replace iodinated proOmpA. To avoid interference of the (pro)OmpA-F-Mal signal(s) with that of F-Mal-labeled inner membrane proteins

we used proOmpA-AF633 in the translocation assays with F-Mal-labeled SecY(EG) complexes. (AF633 can be detected above 645 nm where the emission of fluorescein is negligible).

NEM or F-Mal modification of SecY(S349C)EG vesicles (C5 domain) completely abolishes proOmpA translocation (Fig. 3B,C, lane 6) and strongly inhibits the proOmpA-stimulated SecA ATPase activity (data not shown). Interestingly, NEM modification of the other mutants has no effect on proOmpA translocation, but labeling of IMVs containing SecY(S11C)EG (domain C1), SecY(S111C)EG (domain C2) and SecY(S431C)EG (domain C6) with the considerably larger probe F-Mal partially inhibits proOmpA translocation (Fig. 3C, lanes 2, 3 and 7). Taken together, these results indicate that particularly cytoplasmic domain C5 is highly sensitive for chemical modification.

3.3. Oxidative crosslinking of SecY within an oligomeric SecYEG complex

Several studies indicated that SecYE(G) protomers form oligomeric complexes. For this reason we investigated if the single cysteine SecY mutants can form disulfide bonds upon oxidation. IMVs were incubated with the reducing agent DTT (Fig. 4A) or with the oxidising agent tetrathionate (S_4O_6) (Fig. 4B), and analysed by SDS–PAGE followed by immunodetection with an antibody raised against SecY. Upon oxidation, SecY mutants S11C, S111C and S431C (in C1, C2 and C6, respectively) gave rise to a pronounced crosslink product of 75 kDa (Fig. 4B, lanes 2, 3 and 7). The formation of this product is reversible by DTT and can be prevented by pretreatment of the cysteines with NEM, indicating that the crosslink is indeed disulfide-mediated (data not shown). As the 75 kDa product did not react with antibodies against SecE and SecG, and the appearance was accompanied by a clear decrease of the SecY band (Fig. 4B, lanes 2 and 7), we conclude that the observed crosslink products represent disulfide-linked SecY dimers. It should be noted that SecY mutants
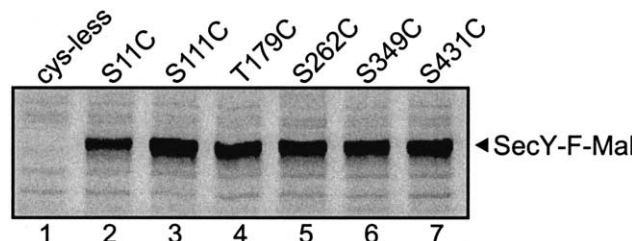

Fig. 2. F-Mal labeling of single cysteine SecY mutants. IMVs containing overproduced single cysteine SecY mutants were incubated with 0.25 mM F-Mal and analysed by 12% SDS–PAGE and in gel UV fluorescence. SecY molecules modified with F-Mal are indicated (SecY-F-Mal).
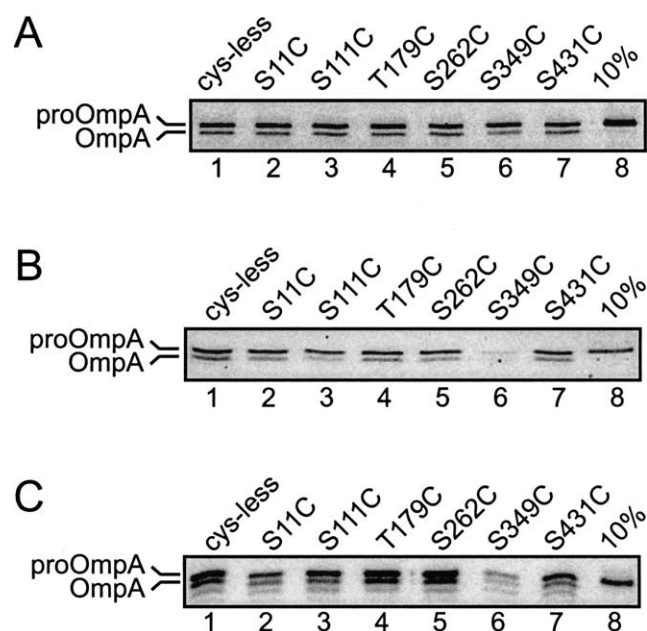
Fig. 3. In vitro translocation activity of NEM and F-Mal-labeled SecYEG complexes. IMVs containing overproduced single cysteine SecY mutants were incubated in the absence (A) and presence of 1 mM NEM (B) or 0.25 mM F-Mal (C), re-isolated and assayed for in vitro translocation of fluorescently labeled proOmpA. Translocation reactions were allowed to proceed for 7 min at 37°C and untranslocated proOmpA was digested by proteinase K. Protease-protected material was separated by 12% SDS–PAGE and visualised by in gel UV fluorescence. ProOmpA-F-Mal was used in (A) and (B), proOmpA-AF633 was used in (C). Lanes 8 represent 10% of the total amount of fluorescent proOmpA added to the translocation reactions.

S11C and S431C even form dimers without S_4O_6 that cannot be completely dissociated by reduction with DTT (Fig. 4A, lanes 2 and 7). This suggests that both the N- and C-termini of neighbouring SecY molecules are in close proximity of each other and therefore crosslink efficiently.

3.4. ProOmpA translocation into IMVs containing disulfide-crosslinked SecY

To investigate whether disulfide-linked SecY dimers are still functional we assayed the oxidised SecY mutants for in vitro translocation activity of proOmpA under non-reducing conditions (OX). As a control we re-reduced the oxidised mutants with DTT and performed translocation reactions under reducing conditions (RR). IMVs containing Cys-less SecYEG complexes are equally active in proOmpA translocation under non-reducing as under reducing conditions (Fig. 5, lanes 1 and 2). This shows that a direct comparison of the translocation activities under both conditions is legitimised. Oxidation of SecY mutants S11C and S431C (domains C1 and C6) does not affect the translocation activity (Fig. 5, lanes 3, 4 and 7, 8), indicating that SecY molecules dimerised via the N- or C-termini are still active. In contrast, oxidation of SecY mutant S111C (domain C2) leads to a drastic inhibition of the translocation (Fig. 5, lane 5), demonstrating that translocation of proOmpA is not allowed into IMVs containing SecY molecules disulfide-crosslinked via S111C. Translocation activity can be restored upon cleavage of the disulfide bond with DTT, showing that crosslinking via SecY(S111C) only transiently inactivates SecYEG (Fig. 5, lane 6).

3.5. Chemical crosslinking of SecY to SecG

Whereas oxidative crosslinking can only provide information on close proximity of two cysteine residues, chemical crosslinking can demonstrate many more intermolecular contacts. We made use of the heterobifunctional crosslinker S-MBS to probe for additional interactions around the introduced cysteines. IMVs overexpressing the mutant SecYEG complexes were incubated with S-MBS and analysed by SDS–PAGE followed by immunodetection with anti-SecY antibodies. SecY(S111C) and SecY(T179C) gave rise to a 50 kDa crosslink product (Fig. 6A, lanes 3 and 4). To identify the product we analysed the samples by anti-SecE (data not shown) and anti-SecG immunodetection (Fig. 6). Only the SecG antibodies reacted with the 50 kDa product formed upon crosslinking of SecY mutants S111C and T179C (Fig. 6B, lanes 2 and 3), confirming the identity of both products as SecG crosslinked to SecY. This indicates that SecY domains C2 and C3 are in close proximity of SecG. Due to the low efficiency of crosslinking we cannot determine the effect of SecY–SecG crosslinking on functionality of the SecYEG complex.

4. Discussion

In this study we have investigated intermolecular interactions at the cytoplasmic side of *E. coli* translocase by means of
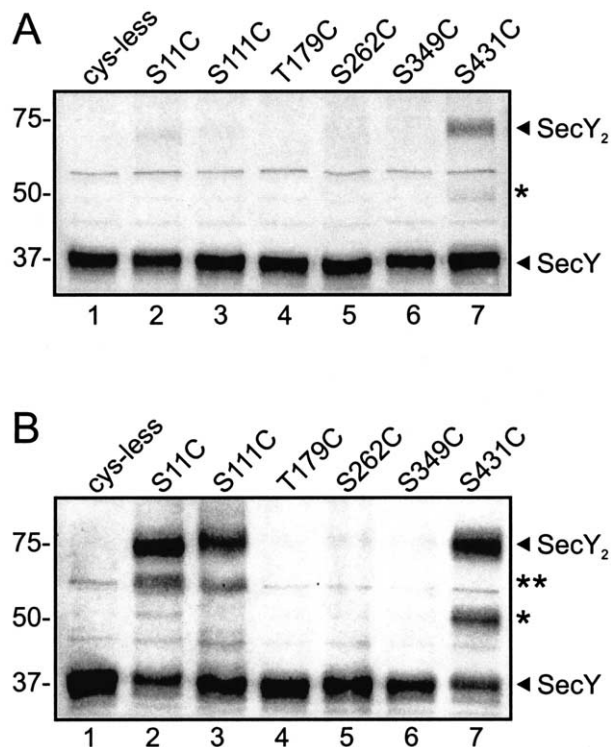

Fig. 4. Disulfide crosslinking of SecY. IMVs containing overproduced single cysteine SecY mutants were reduced with 5 mM DTT (A) or oxidised with 1 mM tetrathionate (S_4O_6) (B). Samples were analysed by 10% SDS–PAGE followed by immunodetection with antibodies raised against SecY. SecY monomers and SecY dimers ($SecY_2$) are indicated. The broad bands indicated by asterisks represent full length SecY disulfide-linked to a 14 kDa C-terminal (*) or 24 kDa N-terminal (**) fragment of SecY [37]. The sharp band just below (**) in A and B is an aspecific cross-reaction of the SecY antibodies.
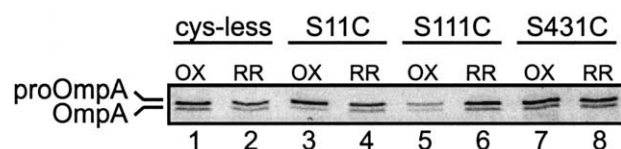
Fig. 5. Translocation activity of oxidised and re-reduced SecYEG complexes. SecY mutants capable of forming SecY dimers were assayed for in vitro translocation activity of F-Mal-labeled proOmpA as described in the legend to Fig. 3. Translocation reactions were performed with oxidised membranes under non-reducing conditions (OX), or with re-reduced membranes under reducing conditions (RR) (for details see Section 2).

thiol chemistry experiments. In each cytoplasmic domain of SecY we have introduced a single cysteine that is accessible for thiol reactive probes and does not interfere with SecYEG functionality. This provides us with a clean system to study the effect of structural changes in these domains caused by chemical modification or crosslinking.

Cytoplasmic domain C5 of SecY is highly conserved and based on studies with cold-sensitive SecY mutants, this region has been proposed to be involved in an interaction with SecA [18,19,38]. Chemical modification of SecY(S349C) in C5 with NEM or F-Mal completely inhibits protein translocation (Fig. 3). This further stresses the importance of this domain for functionality and in contrast to the previous mutagenesis studies we can exclude that the aberrant behaviour observed here is caused by misfolding of the domain during biogenesis since the unmodified mutant is fully functional. Most likely, chemical modification of SecY(S349C) disturbs the previously proposed interaction site with SecA and thus prevents protein translocation.

Despite extensive trials with each of the mutants using different cysteine-specific crosslinkers and crosslinking conditions, we were unable to observe any SecY crosslinks to SecE, SecA or other proteins (E.O. van der Sluis, unpublished results). The sensitivity for chemical modification of SecY(S349C) can explain why we were unsuccessful in observing a SecY–SecA crosslink. First, the presence of a crosslinker on SecY would disturb the interaction with SecA. Second, binding of SecA to SecYEG would prevent the reaction of the crosslinker with the cysteine, making this strategy 'suicidal'.

An oligomeric organisation of SecYEG has been shown with various techniques, but an interaction between two SecY molecules was so far only demonstrated with an intervening SecE dimer [8]. In this study we show that cysteine residues in cytoplasmic domains C1, C2 and C6 of SecY each have the ability to form a disulfide bond with a neighbouring SecY molecule (Fig. 4). Although it could be inferred that the dimers we observe are caused by aspecific interactions between randomly colliding (monomeric) SecYEG protomers, we think this is unlikely for the following reasons: first, we have previously shown that disulfide crosslinking within oligomeric SecYEG complexes is not induced by overexpression as it also occurs at wild-type levels [8]; second, disulfide crosslinking of SecY(S11C) and SecY(S431C) occurs spontaneously whilst membranes are kept at 4°C which is far below the phase transition temperature of the E. coli cytoplasmic membrane and thus minimises lateral diffusion of proteins [39]; third, disulfide crosslinking is not observed with cysteine mutants located in domains C4 and C5 which are of similar

size as C2 suggesting that the crosslinking is not merely caused by high flexibility of these large domains. From this we conclude that the observed SecY dimers truly reflect close spatial arrangements within an oligomeric SecYEG complex.

From the effect of disulfide crosslinking on translocation activity we can obtain more insight in the individual contributions of these domains to protein translocation. SecY crosslinked via S11C or S431C (domains C1 and C6) is equally active as uncrosslinked SecY (Fig. 5). This shows that disulfide crosslinking of SecY per se does not inactivate SecYEG and that during the catalytic cycle of translocation, two SecY N- or C-termini are allowed to remain in close proximity. This constitutive proximity is reflected by the spontaneous oxidation to disulfides observed with SecY mutants S11C and S431C and is in agreement with an oligomeric organisation of SecYEG during translocation as previously proposed [4,10]. It should be noted that a disulfide bond between two N- or C-termini only leads to the formation of a loop structure that can still possess substantial conformational freedom. In contrast, a disulfide bond connecting two genuine loops causes a much greater restraint on flexibility. Indeed, disulfide crosslinking of two SecY C2 domains inactivates the SecYEG complex, showing that dynamics of these domains are required during translocation (Fig. 5). Previous experiments have shown that disulfide crosslinking of two SecE molecules [7] or of SecY to SecE [8,16] inhibits protein translocation. The transmembrane segment at the heart of these interactions (TMS3 of SecE) is located at the interface of two SecYEG
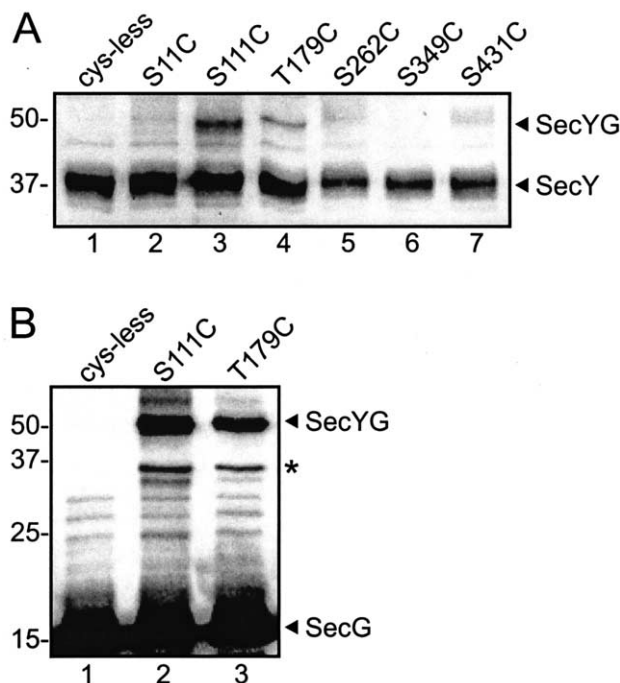

Fig. 6. Chemical crosslinking of SecY to SecG. IMVs containing overproduced single cysteine SecY were incubated with 1 mM sulfo-MBS (S-MBS) and analysed by 10% SDS–PAGE followed by immunodetection with antibodies raised against SecY (A). Single cysteine SecY mutants giving rise to a 50 kDa crosslink product were analysed by immunodetection with antibodies raised against the C-terminus of SecG. SecG and SecY crosslinked to SecG (SecYG) are indicated (B). The asterisk indicates SecG crosslinked to an N-terminal fragment of SecY.

protomers [8]. One of the transmembrane segments anchoring the C2 domain (TMS2 of SecY) has been shown to contact TMS3 of SecE directly [8]. We therefore propose that the C2–C2 disulfide bridge spans the same interface between two SecYEG protomers and that it inactivates SecYEG in a similar manner as the SecE–SecE and the SecY–SecE crosslinks.

Interestingly, all three SecY mutants capable of forming disulfide-linked homodimers (S11C, S111C and S431C in domains C1, C2 and C6, respectively) are moderately sensitive for chemical modifications. NEM modification has no effect on functionality and F-Mal only partially inactivates these mutants (Fig. 3). This suggests that the nature of the interaction disturbed upon modification of the latter mutants is different from that leading to complete inactivation of SecY(S349C). Electron microscopy and BN-PAGE studies have revealed that SecYEG is a conformationally dynamic structure that can exist in multiple oligomeric isoforms [4,10]. Furthermore, it was shown that dimeric SecA recruits multiple SecYEG protomers into a tetrameric assembly that was proposed to represent the functional complex [4]. Biochemical studies with aspecific crosslinkers, however, failed to demonstrate inter-protomer crosslinks [10,11,17]. This was explained by the reaction of the crosslinkers with amino acyl side chains involved in the association of SecYEG protomers, thereby disturbing their association [10]. Modification of the cysteines in SecY domains C1, C2 or C6 could lead to a similarly disturbed interaction. The decrease in translocation activity would thus be caused by disturbed assembly of SecYEG protomers into their functional organisation. Since interactions between SecYEG protomers also take place in the membrane region [8] and are expected in the periplasmic region, disturbance of only a cytoplasmic interaction is not likely to completely prevent association of SecYEG protomers and therefore only partial inactivation of these mutants is observed.

Several biochemical studies have shown that SecG associates with SecYE [28,29], but the domains of SecY or SecE involved in an interaction with SecG were unknown. In this study, we show that SecY domains C2 and C3 are in close proximity to SecG. Since SecG is not essential, it is likely to be located at the periphery of a SecYEG protomer. A biochemical indication for such peripheral localisation of SecG is the crosslink to S111C of SecY which is located close to the interface of two SecYEG protomers. Recent BN-PAGE experiments showed that overproduced SecYEG forms dimeric and tetrameric multimers in addition to monomers, whereas SecYE only forms monomers and dimers [10]. These SecG dependent tetrameric multimers appeared more stable and less dynamic than the dimeric SecYEG complexes. In this light it is interesting to observe that SecG crosslinks to a region of SecY involved in SecYEG oligomerisation. Thus, SecG might directly affect the oligomerisation of SecYEG. The dimeric form of SecG could be related to this oligomerisation [32].

Taken together, the data presented in this study further increase our insight in the structure and the function of SecYEG translocase. This will be useful in interpreting medium resolution 3D structures of SecYEG that are expected in the near future [9].

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