The large first periplasmic loop of SecD and SecF plays an important role in SecDF functioning

Nouwen, N; Piwowarek, M; Berrelkamp, G; Driessen, Arnold

Published in:
Journal of Bacteriology

DOI:

IMPORTANT NOTE: You are advised to consult the publisher’s version (publisher’s PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 16-04-2017
The Large First Periplasmic Loop of SecD and SecF Plays an Important Role in SecDF Functioning

Nico Nouwen,* Magdalena Piwowarek, Greetje Berrelkamp, and Arnold J. M. Driessen

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

Received 4 April 2005/Accepted 27 May 2005

A remarkable feature of proteins of the SecD and SecF family involved in protein translocation is that they possess a very large first periplasmic domain. Here we report that this large first periplasmic domain is not required for the SecD-SecF interaction but that it is important for catalyzing protein translocation.

In bacteria, the majority of secretory proteins and membrane proteins are translocated across or inserted into the cytoplasmic membrane by the Sec machinery. In Escherichia coli, the Sec machinery compromises eight proteins, SecA, SecD, SecE, SecF, SecG, SecY, YajC, and YidC (see reference 2 for a review). SecY, SecE, and SecG form a heterotrimeric integral membrane domain with which a peripheral ATPase, SecA, associates to form the core of the translocate complex. SecD, SecF, and YajC form a distinct complex that can associate with the SecYEG complex (4). YidC has been discovered as an integral membrane protein that is in the close vicinity of transmembrane segments that insert into the membrane either independently or via the SecYEG complex (16, 18) YidC can be found associated to both the heterotrimeric SecYEG and SecDF-YajC complexes (12, 16). In analogy with OXA1 in mitochondria, YidC plays a crucial role in the biogenesis of mitochondrial inner-membrane vesicles (IMVs) were isolated, and the expression of plasmid-encoded SecDF-YajC was analyzed by the large periplasmic loop. To study the role of the very large first periplasmic domain (P1) in SecDF functioning, we created a set of in-frame deletions in secD and secF encoding this domain (Fig. 1, corresponding amino acid deletions). As SecD, SecF, and YajC are encoded by a single operon, which likely results in a well-balanced expression of the three proteins, we expressed the different deletion mutants from a plasmid containing the whole yajC-secDF operon under the control of an IPTG (isopropyl-α-D-thiogalactopyranoside)-inducible promoter. To test the functionality, plasmids containing the different deletions mutants were transformed to E. coli strain JP325 [araΔ714 Δ(argF-lac)U169 rpsL150 relA1 thi flb5301deoC1 ptsF25 recA::cat tgt::kan-araC–P_BAD::yajC-secDF (6)]. In this strain, the chromosomal yajC-secDF operon is under the control of the araB promoter. Consequently, strain JP325 expresses only YajC, SecD, and SecF in the presence of arabinose and does not form colonies on LB agar plates in the presence of glucose. Growth on glucose-containing medium, however, can be restored by introducing a plasmid containing the yajC-secDF operon into the strain (the low basal expression from the trc promoter is sufficient to restore growth). Plasmids encoding SecD P1 deletion mutants very weakly restored growth of strain JP325 on glucose LB agar plates at 37°C (SecD199-279 and SecD325-341 and SecD33-151) or did not restore growth at all (SecD199-279 and SecD325-341 and SecD33-151) or did not restore growth at all (SecD199-279 and SecD325-341 and SecD33-151) or did not restore growth at all (SecD199-279 and SecD325-341 and SecD33-151). The weak complementation of mutants SecD199-279 and SecD325-341 and SecD33-151 became worse at lower temperatures. In contrast, the plasmid encoding the SecF P1 deletion mutant (SecFΔ74-101) gave a strong cold-sensitive phenotype. It was perfectly able to restore growth of strain JP325 at 37°C but not at a temperature of 30°C or lower. The results described above did not change upon the overproduction of the different P1 deletion mutants by the addition of 300 μM IPTG to the LB agar plates.

In vivo expression of the SecD and SecF deletion mutants. The introduction of large deletions in proteins may render them unstable. To determine if the different P1 deletion mutants form a stable protein in the membrane, we expressed them under similar conditions as we tested their ability to restore growth of strain JP325 on glucose medium. To this end, chromosomally encoded SecDF-YajC was depleted by growing strain JP325 for six generations on glucose-containing LB medium. Inner-membrane vesicles (IMVs) were isolated, and the expression of plasmid-encoded SecDF-YajC was analyzed by
sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblotting by using antibodies against SecD and SecF. Using plasmid pET545 encoding wild-type SecDF-YajC, this procedure leads to near-chromosomal levels of SecD and SecF (13). Unfortunately, with the plasmids encoding P1 SecD/F deletion mutants, the expression levels of SecD and SecF obtained were lower than those from cells containing the wild-type plasmid (data not shown). These levels could be equalized by induction of the operon with 300 μM IPTG (Fig. 2, lanes 2 to 6). As a consequence, the final SecD and SecF levels in the membrane are higher than those from wild-type cells. As induction with IPTG did not affect the in vivo complementation results and different protein levels would strongly interfere with the analysis of results from functional studies, we decided to use the membranes containing elevated SecD and SecF levels in the remainder of our study. Membranes from cells expressing SecDΔ325-341 and SecDΔ210-419 contained a 60-kDa protein and a 40-kDa protein, respectively (Fig. 2A, lanes 3 and 6). This corresponds well with the calculated molecular size of the corresponding deletion mutant. In contrast, IMVs from cells expressing SecDΔ199-279 (Fig. 2A, lane 4) or SecDΔ33-151 (Fig. 2A, lane 5) contain a protein that is much smaller than expected from the calculated molecular size. As both proteins reacted with antibodies against SecD (data not shown), the results suggest that these two SecD mutants are unstable.

FIG. 1. Schematic representation of different P1 deletions in SecD and SecF and their ability to complement growth of the SecDF-YajC depletion strain JP325. Regions that are conserved are indicated by black boxes. To test the functionality of the deletion, strain JP325 (yajC::yajC-secDF) was transformed with plasmids pET545 (yajC-secDF), pNN230 (yajC-secDΔ325-341F), pNN231 (yajC-secDΔ199-279F), pNN232 (yajC-secDΔ33-151F), pNN233 (yajC-secDΔ210-419F), and pNN235 (yajC-secDFΔ74-101), and analyzed to restore growth on LB-ampicillin (100 μg/ml) plates containing 0.5% glucose (with or without 300 μM IPTG) at different temperatures (37°C, 30°C, and 22°C). ++, growth; +/−, very poor growth; −, no growth; Cs, growth is cold sensitive (normal growth at 37°C and no growth at 30°C or lower).

FIG. 2. Expression of P1 SecD/F deletion mutants in cells from which chromosomally encoded YajC-SecDF was depleted. IMVs were prepared from strain JP325 containing the different P1 deletion plasmids grown for six generations on LB medium containing 0.5% glucose. Expression of the plasmid-encoded gene products has been induced with 300 μM IPTG. IMVs were analyzed by SDS-PAGE and CBB staining (A) and by immunostaining using antibodies against SecG (B). (C) After solubilization of the IMVs with dodecylmaltoside, SecF was purified with Ni-NTA beads, and the copurification of SecD was analyzed by SDS-PAGE and CBB staining. (D) The presence of YajC in the Ni-NTA-purified material was analyzed by SDS-PAGE and silver staining. Positions of mutant SecD and SecF proteins are indicated with asterisks and circles, respectively. wt, wild type.
Membranes from cells expressing a mutant SecF with a 28-amino-acid deletion in the P1 loop contain normal levels of SecD and (mutant) SecF compared to the wild-type control (SecF/H900474-101 [Fig. 2A, lane 7]). It has been reported that SecDF-YajC depletion results in a twofold reduction of SecG (8). However, with our method to deplete chromosomally encoded SecDF-YajC while expressing the SecD and SecF deletion mutants, no changes in the SecG level were observed (Fig. 2B, compare lane 1 with lanes 2 to 7).

SecD and SecF deletion mutants form a stable SecDF-YajC complex. YajC, SecD, and SecF form a stable heterotrimeric complex in detergent solution (4, 12). To determine if the deletions in the P1 loop affect complex formation, IMVs over-producing the different P1 deletion mutants were solubilized with the detergent dodecylmaltoside, and the membrane extract was incubated with Ni-nitrilotriacetic acid (NTA) agarose beads. Under these conditions, wild-type YajC and SecD co-purify with the histidine-tagged SecF (Fig. 2C and D, lanes 2) (12). The same copurification occurs with the membrane extract from cells expressing SecDΔ325-341, SecDΔ210-419, and SecFΔ74-101, indicating that these three mutants form a stable SecDF-YajC complex (Fig. 2C and D, lanes 3, 6, and 7). The unstable SecDΔ199-279 and SecDΔ33-151 mutants do not co-purify with histidine-tagged SecF (Fig. 2C, lanes 4 and 5). Immunoblotting showed that YidC copurifies with both wild-type and mutant SecDF-YajC complexes (data not shown). The Coomassie blue-stained gel with the SecD and SecF mutants indicates that the YidC fraction that co-purifies is small compared to the amount of SecD and SecF in the complex (Fig. 2C, lanes 3 and 6).

Inner-membrane vesicles containing SecD and SecF with a deletion in the large periplasmic loop display an in vitro translocation defect. Thus far, in vitro assays did not reveal a clear stimulatory effect of SecDF-YajC on protein translocation. We observed that when chemical amounts of proOmpA were used, the translocation kinetics into IMVs lacking SecDF-YajC is about twofold faster than that into IMVs containing SecDF-YajC complexes (data not shown). To test the effects of the deletions in the large periplasmic loop, we used the assay described above to determine the initial rates of proOmpA translocation at 37°C and 30°C. All
deletions resulted in a decrease in the rate of proOmpA translocation. For both temperatures and for all deletions in the periplasmic P1 loop of SecD, this decrease is similar to the reduction observed with IMVs that lack the SecDF-YajC complex (Fig. 3C and D). proOmpA translocation into IMVs from SecDΔ74-101 mutant cells shows a cold-sensitive phenotype. Although at 37°C proOmpA translocation into SecFΔ74-101 IMVs is lower than that for wild-type IMVs, translocation is clearly better than that for SecDF-YajC-depleted IMVs. The latter difference, however, is nearly diminished at a low temperature, i.e., 30°C. To exclude the possibility that the obtained translocation defects are caused by a reduced PMF generation, we measured PMF generation with ATP and NADH. With both energy sources, all IMVs were equally active in generating a PMF (data not shown). Thus, the reduced translocation into IMVs from the different mutants is directly caused by the deletion in the P1 loop.

Our study shows that small deletions in P1-SecD and P1-SecF (13 and 28 amino acids, respectively) impaired the SecDF functioning both in vivo and in vitro. As both mutant proteins are expressed and are able to form a stable SecDF-YajC complex, this demonstrates that the periplasmic P1 domain in SecD and SecF fulfills a critical function. Moreover, since a plex, this demonstrates that the periplasmic P1 domain in functioning both in vivo and in vitro. As both mutant proteins SecF (13 and 28 amino acids, respectively) impaired the SecDF complex with SecYEG and SecDF-YajC. With such an assay, the catalytic function of the SecDF-YajC complex in protein translocation could be analyzed in more detail.

This work was supported by the Royal Academy of Sciences of The Netherlands (KNAW).

REFERENCES