Roles of the Periplasmic Domain of *Escherichia coli* FtsH (HflB) in Protein Interactions and Activity Modulation*

(Received for publication, May 18, 1998, and in revised form, June 19, 1998)

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FtsH is a membrane-bound and ATP-dependent protease of *Escherichia coli*, known to degrade SecY, a membrane protein for protein translocation, and CII, a soluble transcription factor for lysis/lysogeny decision of phage λ. FtsH forms a homo-oligomeric complex as well as a hetero-oligomeric complex with HflKC, a putative modulator of FtsH. Although FtsH has a small periplasmic region, HflKC is mostly exposed to the periplasmic space. We studied the roles of the periplasmic region of FtsH by engineering mutations in this protein. FtsHΔ3236, lacking most of the periplasmic region, retained the in vivo ability to degrade SecY but not CII, resulting in high frequency lysogenization of λ. Several insertion mutations in the periplasmic region of FtsH also differentially affected the proteolytic activities of FtsH. Interestingly, purified and detergent-solubilized FtsHΔ3236 was as active as the wild-type enzyme in degrading SecY and CII, although its ATPase activity was lowered 5-fold. Affinity chromatography using histidine-tagged derivatives showed that the periplasmic domain-deleted FtsH no longer interacted with FtsH or HflKC. Although FtsHΔ3236-His<sub>6</sub>-Myc lost the static FtsH-FtsH interaction, it retained the ability to change its conformation in an ATP-dependent manner at 37 °C, leading to a limited oligomerization. These results suggest that the periplasmic region of FtsH has crucial roles in the protein-protein interactions of this complex and in the modulation of its proteolytic functions against different substrates.

Among ATP-dependent proteases of *Escherichia coli*, FtsH is unique in that it is membrane integrated (1) and essential for cell viability (2, 3). Its amino-terminal region includes two transmembrane segments and the flanked periplasmic domain of 72 amino acids, whereas its central region has sequence homology to the AAA family of ATPases, followed by the carboxyl-terminal region having a Zn<sup>2+</sup>-metalloprotease motif (HEXXH) (1, 4). Although importance of the latter two cytoplasmic regions has been well documented (5, 6), the roles of the transmembrane and the periplasmic regions are only poorly understood.

We identified three membrane-integrated substrates of FtsH: SecY subunit of protein translocase (5, 7), subunit α of the F<sub>0</sub>F<sub>1</sub> ATPase (F<sub>0</sub>α) (8), and the YccA protein (9). Elimination of SecY and F<sub>0</sub>α, when they failed to associate with their respective partner proteins, seems to serve as a mechanism to keep the integrity of the membrane (5, 7, 8, 10). FtsH has roles in regulation of gene expression also because it degrades some cytosolic transcription factors such as CII, which determines the lysogeny commitment of phage λ (11–13), and α<sub>32</sub>, which controls the heat-shock response (14, 15).

It is also known that some ftsH mutations cause abnormal translocation of the PhoA moiety attached to the carboxy-terminal cytoplasmic region of SecY (Std phenotype) as well as significant defects in protein export (3, 16). The tolZ allele of ftsH confers resistance to several colicins and defective membrane potential (6, 17). We showed recently that FtsH has a proteolysis-independent polypeptide-binding activity (18). These findings raise a possibility that FtsH acts like a molecular chaperone in protein assembly processes.

FtsH forms homo-oligomeric complexes in which the amino-terminal membrane region interacts (19). It also associates with the HflK-HflKC complex (HflKC) (10). Unlike the prevailing view, HflKC does not itself degrade CII and resides mostly on the periplasmic side of the membrane (12). We isolated mutations hflK13 and hflC9, which interfere with the SecY degradation function of FtsH (10). These mutations stabilize SecY and F<sub>0</sub>α but not CII (9, 12). We also identified a mutation, yccA11, that stabilizes only the membrane-bound substrates of FtsH (9). The YccA protein is a multi-spanning membrane protein that was shown to be a substrate of FtsH both in vivo and in vitro (9). The YccA11 mutant protein, having an internal deletion of 8 amino acids near the amino terminus, binds to FtsH and HflKC but is refractory to degradation. The inhibitory effect of YccA11 on SecY degradation was only observed in the presence of HflKC. The proposed function of HflKC is to differentially modulate the proteolytic activities of FtsH against different classes of substrates (9).

In this work we carried out mutational analyses of the periplasmic region of FtsH. It was found that mutational disruptions of the periplasmic region resulted in the impairment of the FtsH-FtsH and FtsH-HflKC subunit interactions, affecting the in vivo proteolytic activities of FtsH against the same spectrum of substrates as the hflK13, hflC9, and yccA11 mutations.

**MATERIALS AND METHODS**

**Bacterial Strains and Plasmid—*E. coli* strains and plasmids used in this study are listed in Table I. Strain AR3291 (ΔftsH3:kan) was a derivative of W3110 bearing a suppressor sfhC21 with a linked transposon zod-220::Tn10.**

**∥** pSTD233 was a derivative of pSTD120 (19) from which the two BamHI sites were eliminated (for the Taphoavin analysis


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*This work was supported by grants from the Ministry of Education, Science, Sports and Culture, Japan (to Y. A., T. O., and K. I.), from CREST, Japan Science and Technology Corp. (to K. I.), and from the Human Frontier Science Program Organization (to K. I.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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TABLE I

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype or enzyme</th>
<th>Reference or source</th>
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<tr>
<td>AK519</td>
<td>Δlac-pro/F’ lacP/lacZ-Δ15</td>
<td>7</td>
</tr>
<tr>
<td>AK525</td>
<td>Δlac-pro/zgi-525::IS1A/lacP/lacZ-Δ15</td>
<td>7</td>
</tr>
<tr>
<td>AK900</td>
<td>ΔhisF-Δlac/lacZ-Δ15</td>
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</tr>
<tr>
<td>AD368</td>
<td>ftsH11/F’ lacP/lacZ-Δ15</td>
<td>5</td>
</tr>
<tr>
<td>YE024</td>
<td>ompT::kan/F’ lacP/lacZ-Δ15</td>
<td>5</td>
</tr>
<tr>
<td>AR2391</td>
<td>ΔftsH13::kan, sftC</td>
<td>21</td>
</tr>
</tbody>
</table>

Plasmids

- pKY258: secY-lacZa
- pKY321: secY-phoA C6
- pSTD113: ftsH-his6-myc
- pSTD233: ftsH-his6-myc on pMW119
- pSTD236: ftsH236-his6-myc version of pSTD233
- pSTD240: secY, hffX-hfF-hfC9
- pSTD243: ftsH236-his6-myc version of pSTD113
- pNS104: ftsH104-his6-myc version of pSTD233
- pNS105: ftsH105-his6-myc version of pSTD233
- pMW119H: pMW119 with a frame shift in lacZa

Described below; site-direct mutagenesis (20) using a mutagenic primer, ATTTCGCGGTCGTGAAC, eliminated the one within ftsH-his6-myc without altering the amino acid sequence, whereas the one in the multicloning region was eliminated by digestion with BamHI, filling in with T4 DNA polymerase, and self-ligation. pSTD236 was constructed from pSTD233 using Quick Change mutagenesis kit (Stratagene) and primers CTTCTGTCCTCGCCGTTCGCTCCGTCATTAGACTCGCTGGCC and GCCGGAGGGAGTCTAATGCGCTACGAGCCTCGAAGACCAAG. pSTD243 was constructed by replacing a 1.2-kilobase XbaI-MluI fragment of pSTD113 (19) with the corresponding fragment of pSTD236. pMW119H was constructed from pMW119 by treatment with HindIII and T4 DNA polymerase, followed by self-ligation. pSTD240 was constructed by cloning an EcoRI-HindIII fragment of plac-ΔlacZa (12) into pET28 (Takara Shuzo). For constructing pSTD241, a 3.9-kilobase KpnI-EcoRI fragment containing the hfX-hfF-hfC9 genes was first cloned into pET29 (Takara Shuzo); then, the HindIII secY fragment of pKY321 (21) was inserted into the HindIII site on the vector.

Insertion Mutagenesis—Insertion mutations in the periplasmic domain of FtsH were isolated by the TagphoA/in method (22). TagphoA/in was allowed to transpose onto pSTD235, and periplasmic insertions were converted into those of an in-frame 31-codons by excision of a BamHI fragment (22). Exact insertion points were determined by DNA sequencing (Fig. 4). Plasmids carrying insertion 104 and insertion 105 were designated as pNS104 and pNS105, respectively.

Media—L medium (23), peptone medium (24), and M9 medium (23) were used. Ampicillin (50 μg/ml) and/or chloramphenicol (20 or 100 μg/ml) were added for growing plasmid-bearing strains.

Pulse-chase and Immunoprecipitation Experiments—Cells were pulse labeled with [35S]methionine and chased as described previously (7). Whereas CII was directly analyzed by 15% SDS-PAGE, 2 SecY was published procedures (27, 28). Labeled proteins were immunoprecipitated (25) and separated by 15% acrylamide, 0.12% N,N'-methylene-bis-acrylamide SDS-PAGE (26). Labeled proteins were visualized and quantified by a Fuji BAS2000 imaging analyzer.

Roles of the FtsH Periplasmic Domain

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2 The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; IPTG, isopropyl-β-D-thiogalactopyranoside; NTA, nitrilotriacetic acid.
Roles of the FtsH Periplasmic Domain

Table II

<table>
<thead>
<tr>
<th>Strain</th>
<th>Lysogenization frequency</th>
</tr>
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<tr>
<td>AR3291/pMW119H</td>
<td>100</td>
</tr>
<tr>
<td>AR3291/pSTD233</td>
<td>4.3</td>
</tr>
<tr>
<td>AR3291/pSTD236</td>
<td>80</td>
</tr>
<tr>
<td>AR3291/pINS104</td>
<td>15</td>
</tr>
<tr>
<td>AR3291/pINS105</td>
<td>4.5</td>
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</table>

*Lysojenization frequency of λ was determined as described previously (12).*

Fig. 2. In vivo degradation of the σCII protein by FtsHΔ236-His6-Myc. Cells of AK525 carrying pKY240 (ΔII) and pSTD236 (squares), pSTD233 (diamonds), or pMW119H (circles) were grown in M9 medium, induced with 1 mM IPTG and 5 mM cAMP for 1 h, and pulse labeled with [35S]methionine for 30 s, followed by chase for the indicated periods. Proteins of a fixed total radioactivity were subjected to SDS-PAGE. Radioactivities associated with CII were determined, and values are reported as % of the initial (0-min chase) radioactivity for each culture.

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(A3291) bearing pSTD233 (ftsH-his6-myc), pSTD236 (ftsHΔ236-his6-myc), or pMW119H (vector) as hosts for λ phage infection (Table II). Although ftsH is essential for bacterial growth, a suppressor mutation, sfhC, in AR3291 enables cell growth in the absence of FtsH (6). The strain AR3291 itself showed a lysogenization frequency of almost 100%. When FtsH-His6-Myc was synthesized from pSTD233 in the same strain, lysogenization frequency was decreased to 4.3%. In contrast, the expression of ftsHΔ236-his6-myc from pSTD236 affected the lysogenization frequency only to a small extent (to about 80%). Consistent results were obtained by scoring sensitivity of these bacteria to λc17, which cannot propagate in cells of increased CII contents (33). Whereas AR3291/ftsH-his6-myc was sensitive to λc17, AR3291/ftsHΔ236-his6-myc allowed far less plaque formation. These results, taken together, suggest that FtsHΔ236-His6-Myc has greatly reduced ability to degrade the CII protein.

Direct examination of intracellular stability of CII by pulse-chase experiments (Fig. 2) showed that its half-life in the zgj-525:IS1A cells (with lowered FtsH content) was about 23 min at 37°C. Although the half-life of CII was shortened to 12 min in the presence of the ftsH-his6-myc plasmid, the ftsHΔ236-his6-myc plasmid did not significantly affect the degradation kinetics of CII. We conclude that FtsHΔ236-His6-Myc is almost inactive in degrading the CII protein in vivo.

Effects of FtsHΔ236-His6-Myc on the Std Phenotype—Impairment of the FtsH functions causes abnormal periplasmic localization of the PhoA moiety attached to the carboxyl-terminal cytoplasmic region of SecY. This phenotype, termed STD, can be assessed by measuring alkaline phosphatase activity as well as examining trypsin sensitivity of the PhoA moiety (3). The zgj-525:IS1A mutant expressing SecY-PhoA C6 fusion had the PhoA activity that was at least 2.5-fold higher than that in the

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Fig. 1. In vivo degradation of SecY by FtsHΔ236-His6-Myc. A, strain AK519 (ftsH+) was transformed with pKY258 (secY-lacZa) and pMW119H (vector, lane 1). AK525 (zgj-525:IS1A) were transformed with pKY258 and pMW119H (lane 2), pSTD233 (ftsH-his6-myc, lane 3), pSTD236 (ftsHΔ236-his6-myc, lane 4), pINS104 (ftsHΔ104-his6-myc, lane 5), or pINS105 (ftsHΔ105-his6-myc, lane 6). Cells were grown in peptone medium and induced with 1 mM IPTG for 1 h. β-Galactosidase (LacZ) activities are presented as relative activities to that of AK519/pKY258/pMW119H. Vec, WT, +, and Cs indicate a vector plasmid, the wild-type ftsH-his6-myc gene on the vector, the wild-type ftsH gene on the chromosome, and the zgj-525:IS1A mutation on the chromosome, respectively. B, cells of AK525 carrying pKY258 and pSTD236 (squares), pSTD233 (diamonds), or pMW119H (circles) were grown in M9 medium, induced with 1 mM IPTG and 5 mM cAMP for 1 h, pulse labeled with [35S]methionine for 30 s, and chased for the indicated periods. Proteins of a fixed total radioactivity were precipitated with trichloroacetic acid, and SecY-LacZa was immunoprecipitated. Radioactivities associated with SecY-LacZa were determined after SDS-PAGE. Values are reported as % of the initial (0-min chase) radioactivity for each culture.

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AK519/pKY321/pMW119H were depicted. Using anti-PhoA (5 prime Inc.). PhoA* indicates the trypsin-resistant PhoA fragment.

Roles of the FtsH Periplasmic Domain

Fig. 3. Activity of FtsHΔ236-His6-Myc to complement the Std phenotype. A, strain AK519 was transformed with pKY321 (secY-phoA6) and pMW119H (lane 1). AK525 was transformed with pKY321 and pMW119H (lane 2), pSTD233 (lane 3), pSTD236 (lane 4), pINS104 (lane 5), or pINS105 (lane 6). Cells were grown in peptone medium and induced with 1 mM IPTG for 1 h. PhoA activities relative to that of AK519/pKY321/pwtW119H were depicted. B, cells of AK525 carrying pKY321 and pSTD236 (lanes 1 and 2), pSTD233 (lanes 3 and 4) or pMW119H (lanes 5 and 6) were grown in peptone medium, induced with 1 mM IPTG for 2 h. Cells were disrupted by lysozyme-freezing-thawing and treated with or without trypsin (50 μg/ml) as indicated. Proteins were separated by SDS-PAGE and visualized with immunoblotting using anti-PhoA (5 prime → 3 prime Inc.). PhoA* indicates the trypsin-resistant PhoA fragment.

Self-interaction of FtsH Is Impaired by the Periplasmic Deletion—FtsH forms a homo-oligomeric complex, and this FtsH-FtsH interaction is mediated by the amino-terminal region (16, 19). We examined interaction between FtsH-His6-Myc and FtsH. To minimize possible complication arising from the FtsH-HflKC interaction, we used the TnphoA/in method (22). We identified four different insertions within the periplasmic region from a total of 10 independent isolates (Fig. 4). They were classified into two groups: class I complementing theftsH1 mutation and class II without complementing activity. It was found that class I mutations had occurred after the 26th or 34th codon, whereas the class II mutations had occurred after the 42nd or 60th codon (Fig. 4). All the insertion derivatives were identified by immunoblotting experiments using anti-FtsH or anti-Myc antibodies as proteins of slightly slower electrophoretic mobilities than FtsH-His6-Myc (data not shown). We chose 105 (class I) and 104 (class II) for further analyses.

These insertion derivatives lowered the β-galactosidase activity of SecY-LacZ (Fig. 1A, columns 5 and 6) as well as the PhoA activity of SecY-PhoA C6 (Fig. 3A, columns 5 and 6) in the zgi-525::IS4A mutant cells to the wild-type level. 104 only weakly complemented the λ lysogenization phenotype; it lowered the lysogenization frequency in theftsH-disrupted cells to 15% (Table II) and made AR3291 (∆ftsH3::kan) moderately sensitive to αc17. In contrast, 105 lowered the lysogenization frequency to 4.5% (Table II) and made AR3291 fully sensitive to αc17. We also examined the remaining insertion mutations. All of the class II mutants were defective in degradation of CII (αc17-resistant) but had nearly wild-type levels of SecY-degradation activity (white colonies on plates containing 5-bromo-4-chloro-3-indoryl-β-D-galactoside). Thus, class II periplasmic insertion mutations selectively affect degradation of CII.

Isolation and Characterization of Insertion Mutations Affecting the Periplasmic Region of FtsH—We isolated a series of 31 amino acid-insertion mutations in the periplasmic region of FtsH-His6-Myc using the TnphoA/in method (22). We identified four different insertions within the periplasmic region from a total of 10 independent isolates (Fig. 4). They were classified into two groups: class I complementing theftsH1 mutation and class II without complementing activity. It was found that class I mutations had occurred after the 26th or 34th codon, whereas the class II mutations had occurred after the 42nd or 60th codon (Fig. 4). All the insertion derivatives were identified by immunoblotting experiments using anti-FtsH or anti-Myc antibodies as proteins of slightly slower electrophoretic mobilities than FtsH-His6-Myc (data not shown). We chose 105 (class I) and 104 (class II) for further analyses.

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tagged derivative. In contrast, the chromosomally encoded FtsH was not detected in the eluate fraction when the sample from the cells expressing FtsHΔ236-His<sub>6</sub>-Myc was used, although FtsHΔ236-His<sub>6</sub>-Myc itself was normally recovered in this fraction (Fig. 5B). These results indicate that the deletion of the periplasmic region impairs the self-interacting property of FtsH.

**FtsH-HflKC Interaction Is Impaired by the Periplasmic Deletion**—FtsH forms a complex with HflKC (10), which can also be isolated using His<sub>6</sub>-tagged FtsH (Fig. 6A). The ΔftsH strain was used as a host to avoid any contribution from the chromosomally encoded FtsH. FtsHΔ236-His<sub>6</sub>-Myc failed to bring down HflKC (Fig. 6B). Thus, the periplasmic domain of FtsH seems to be important for the FtsH-HflKC association.

We previously showed that the products of the hflK13 and hflC9 mutant genes interfere with the SecY-degrading function of FtsH (see Introduction). We examined whether HflC9 interferes with the FtsHΔ236-His<sub>6</sub>-Myc-mediated degradation of SecY. A plasmid (pSTD241) expressing SecY, HflK and HflC9 was introduced into strain AK990 (ΔhflK::kan) bearing a compatible low copy plasmid carrying either ftsH-his<sub>6</sub>-myc or ftsHΔ236-his<sub>6</sub>-myc. Overproduced SecY remained stable even if FtsH-His<sub>6</sub>-Myc was overproduced (Fig. 7, diamonds), indicating that excess HflK-HflC9 inhibited FtsH-His<sub>6</sub>-Myc. On the other hand, when FtsHΔ236-His<sub>6</sub>-Myc was expressed, SecY was markedly destabilized (Fig. 7, squares), indicating that FtsHΔ236-His<sub>6</sub>-Myc was refractory to the inhibition by HflK-HflC9. We conclude that interaction between FtsH and HflKC is impaired by the deletion of the FtsH periplasmic region.

**In Vitro Activities of FtsH**—We purified FtsHΔ236-His<sub>6</sub>-Myc (Fig. 8A). It was found that FtsHΔ236-His<sub>6</sub>-Myc (0.71 μmol P<sub>i</sub> released/mg protein/h) was only about 1/5 as active as the wild-type protein (4.1) in ATP hydrolysis.

Proteolytic activities against purified SecY as well as against [<sup>35</sup>S]methionine-labeled CII were examined. FtsHΔ236-His<sub>6</sub>-Myc was as active as FtsH-His<sub>6</sub>-Myc in degrading not only SecY (Fig. 8B) but also CII (Fig. 8C). These proteolytic activities were all ATP dependent. It should be noted again that FtsHΔ236-His<sub>6</sub>-Myc has markedly reduced ATPase activity and that it is devoid of the CII degradation function in vivo.

The apparent uncoupling of the proteolytic activities of FtsHΔ236-His<sub>6</sub>-Myc from the ATPase activity prompted us to examine whether this mutant protein undergoes the ATP-dependent conformational change as was observed for the wild-type protein (18). In the presence of ATP, a trypsin-resistant FtsH fragment of 33 kDa was generated both for FtsHΔ236-His<sub>6</sub>-Myc (Fig. 9) and FtsH-His<sub>6</sub>-Myc (18). Thus, the deletion of the periplasmic region does not abolish the ATP-dependent structural change.

We then studied behaviors of FtsH-His<sub>6</sub>-Myc and FtsHΔ236-His<sub>6</sub>-Myc in gel filtration chromatography under various conditions (Fig. 10). FtsH-His<sub>6</sub>-Myc was mainly eluted at fractions 16–18 (molecular mass of about 1000 kDa as calibrated with soluble protein markers; Fig. 10A), consistent with an oligomeric structure. Incubation with ATP at 0 °C did not affect the elusion profile (Fig. 10B). Pre-incubation at 37 °C in the absence of ATP rendered FtsH-His<sub>6</sub>-Myc unrecoverable from any fractions (Fig. 10C). As the formation of non-filterable aggregates of FtsH was demonstrated previously (18), the above results should have been due to entrapment of aggregated FtsH-His<sub>6</sub>-Myc by the prefiltration device of the column. ATP
Roles of the FtsH Periplasmic Domain

FIG. 8. In vitro activities of FtsHΔ236-His6-Myc to degrade SecY and CII. A, purified preparations of FtsH-His6-Myc (0.475 μg; lane 2) and FtsHΔ236-His6-Myc (0.55 μg; lane 3) were visualized by SDS-PAGE and staining with Coomassie Brilliant Blue. Lane 1 was for molecular weight standards. Note that the faster migrating protein in lane 2 was a carboxyl-terminally truncated form of FtsH-His6-Myc (FtsHΔ) that had lost the bipartite tag (19). B, a purified preparation of SecY (40 ng) was incubated at 37 °C with FtsH-His6-Myc (500 ng; lanes 1–10) or FtsHΔ236-His6-Myc (500 ng; lanes 11–20) in the presence (lanes 6–10 and 16–20) or absence (lanes 1–5 and 11–15) of 3.3 mM ATP. Portions of the samples were withdrawn at 0 (lanes 1, 6, 11, and 16), 7.5 (lanes 2, 7, 12, and 17), 15 (lanes 3, 8, 13, and 18), 30 (lanes 4, 9, 14, and 19), and 60 (lanes 5, 10, 15, and 20) min. Proteins were separated by SDS-PAGE and visualized by immunoblotting using ant-FtsH serum. C, partially purified and [35S]methionine-labeled CII was incubated at 37 °C with FtsH-His6-Myc (500 ng; lanes 1–8) or FtsHΔ236-His6-Myc (500 ng; lanes 9–16) in the presence (lanes 5–8 and 13–16) or absence (lanes 1–4 and 9–12) of 3.3 mM ATP. Portions of the samples were withdrawn at 0 (lanes 1, 5, 9, and 13), 0.5 (lanes 2, 6, 10, and 14), 1 (lanes 3, 7, 11, and 15) and 2 (lanes 4, 8, 12, and 16) h. Proteins were separated by SDS-PAGE and visualized by BAS2000 imaging analyzer. SecY′ indicates an amino-terminal fragment of SecY present in the purified sample (5).

FIG. 9. Trypsin digestion patterns of FtsHΔ236-His6-Myc in the presence or absence of ATP. FtsHΔ236-His6-Myc (13.2 μg) was incubated with 5 μg/ml trypsin at 0 °C for 0 (lanes 1 and 6), 2 (lanes 2 and 7), 4 (lanes 3 and 8), 8 (lanes 4 and 9), and 16 (lanes 5 and 10) min, in the presence (lanes 6–10) or absence (lanes 1–5) of 1 mM ATP. Proteins were separated by 16.1% SDS-PAGE and visualized by immunoblotting using ant-FtsH. The arrowhead indicates a trypsin-resistant 33-kDa fragment of FtsH.

prevented this aggregation of FtsH-His6-Myc (Fig. 10D) as shown previously (18); an FtsH species was generated that eluted slightly earlier (fractions 14–16).

FtsHΔ236-His6-Myc was found at fractions (19–21) corresponding to about 200 kDa (Fig. 10E). Again, pre-incubation with ATP at 0 °C did not affect the elution profile (Fig. 10F). In contrast to FtsH-His6-Myc, FtsHΔ236-His6-Myc did not aggregate extensively, even when pre-incubated at 37 °C in the absence of ATP. Although some limited extent of oligomerization was observed for a fraction of FtsHΔ236-His6-Myc, the bulk of it largely retained the original elution profile (Fig. 10G). These results indicate again that FtsHΔ236-His6-Myc is defective in the FtsH-FtsH interaction. However, when FtsHΔ236-His6-Myc was pre-incubated with ATP at 37 °C, a new form of FtsHΔ236-His6-Myc was found (fractions 15–20; Fig. 10H). This elution profile indicates some oligomerization. Adenosine-5′-o-(3-thiotriphosphate) gave similar results (data not shown). These results suggest that ATP induces periplasmic domain-independent association of FtsH.

DISCUSSION

We have shown here that the periplasmic domain of FtsH is important for its function. The growth-supporting function of FtsH requires the intact periplasmic domain. The periplasmic region of FtsH is not essential for the in vivo proteolytic activity of FtsH against SecY, but it is required for in vivo degradation of CII. It was shown that FtsH-HIFK interaction is disrupted by the lack of the periplasmic domain. Although the oligomeric interaction of FtsH is also disrupted, the substrate-specific inactivation of FtsHΔ236-His6-Myc may primarily be ascribed to the lack of interaction with HIFK. This is because the ΔhflKC mutant, in which the homo-oligomeric interaction of FtsH is maintained,3 exhibits similar deviation in substrate preference of FtsH.

In apparent contradiction to the in vivo results, FtsHΔ236-His6-Myc in detergent extracts can degrade both SecY and CII. Thus, FtsHΔ236-His6-Myc retains intrinsic proteolytic activity

even against CII. We suspect that the lack of interaction with HflKC may be responsible for the in vivo inability of FtsHΔ236-His$_6$-Myc to degrade CII. In contrast, in vitro conditions in which any topological segregation has been compromised seem to allow the mutant protein to act against CII. We proposed the following mechanism about the ΔhflKC effect in vivo (12). HflKC normally acts from the periplasmic side as a negative modulator of proteolysis of membrane-bound substrates. In its absence, FtsH is directed more to the membrane-bound substrates, and soluble protein substrates might escape from FtsH. This “balance shift” model may also explain the phenotypes of FtsHΔ236-His$_6$-Myc, which cannot interact with HflKC. Other explanations may also be possible. For instance, anchoring of FtsH to the membrane may restrict accessibility of substrates to FtsH from the cytoplasmic side. In this case, HflKC will somehow make FtsH overcome such a restriction.

HflK and HIC have large periplasmic domains of about 35 kDa (12), whereas the cytoplasmic tail of HIK is only 79 residues long, and HIC has essentially no cytoplasmically exposed region (12). Deletion of the cytoplasmic region of HIK does not abolish the FtsH-HIK interaction. Thus, the cytoplasmic domains of HflK and HIC do not significantly contribute to their interaction. It is interesting to know whether the transmembrane segments of FtsH and HIKC interact. Our results indicate that transmembrane interaction alone, if any, is not sufficient.

Although FtsHΔ236-His$_6$-Myc was only about 20% active in ATP hydrolysis, it exhibited almost full proteolytic activities in vitro. The ATP-induced conformational change is unaffected by the Δ236 mutation (Fig. 9). The results of Ni-NTA affinity isolation and gel filtration experiments suggest that FtsHΔ236-His$_6$-Myc in isolation in the absence of ATP exists as a monomeric form. However, ATP induces some oligomerization of FtsHΔ236-His$_6$-Myc at 37 °C. Thus, although the periplasmic region of FtsH is important for the stable FtsH-FtsH interaction, some other parts in FtsH can undergo ATP-dependent self-association. It is not known whether FtsH can have proteolytic activity in a monomeric state. Unlike the wild-type FtsH that makes extensive aggregates upon incubation at 37 °C in the absence of ATP (Fig. 10) (18), FtsHΔ236-His$_6$-Myc did not form such aggregates. Thus, in the wild-type protein, ATP may be partly utilized to prevent the formation of the aggregates, but this ATP requirement is alleviated for FtsHΔ236-His$_6$-Myc, providing a possible explanation for its low ATPase activity accompanied by high protease activity. It should be noted that FtsHΔ236-His$_6$-Myc still requires ATP hydrolysis to catalyze proteolysis. Because neither binding nor hydrolysis of ATP is required for the substrate binding of FtsH (18), the role of ATP hydrolysis will be in a post-binding processes, in which substrate proteins are presented to the proteolytic active site.

Acknowledgments—We thank H. Tokuda for a gift of the purified SecY protein, C. Herman for a gift of ptac-cII Y42, and T. Yabe, Y. Shimizu, and K. Mochizuki for technical and secretarial assistance.

REFERENCES


FIG. 10. Gel filtration profiles of FtsHΔ236-His$_6$-Myc and FtsHΔ236-His$_6$-Myc. WT, wild type.