Roles of Homooligomerization and Membrane Association in ATPase and Proteolytic Activities of FtsH in Vitro†

Yoshinori Akiyama* and Koreaki Ito

Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan

Received January 5, 2001; Revised Manuscript Received March 28, 2001

ABSTRACT: *Escherichia coli* FtsH is a membrane-bound and ATP-dependent protease which degrades some soluble and integral membrane proteins. The N-terminal region of FtsH mediates membrane association as well as homooligomeric interaction of this enzyme. Previously, we studied in vivo functionality of FtsH derivatives, in which the N-terminal membrane region was either deleted (FtsH-(ΔTM)), replaced by a leucine zipper (Zip-FtsH(ΔTM)), or replaced by a lactose permease transmembrane segment (LacY-FtsH). It was indicated that homooligomerization is required for the minimum proteolytic activity, whereas a transmembrane sequence is required for membrane protein degradation. Here we characterized these proteins in vitro. Although these mutant enzymes were very low in their activities, they were significantly stimulated by dimethyl sulfoxide, which enabled us to characterize their activities. LacY-FtsH degraded both soluble and membrane proteins, but Zip-FtsH(ΔTM) only degraded soluble proteins. These proteins also exhibited significant ATPase activities. However, FtsH(ΔTM) remained inactive both in ATPase and in protease activities even in the presence of dimethyl sulfoxide. The monomeric FtsH(ΔTM) was able to bind ATP and a denatured protein. These results indicate that subunit association is important for the enzymatic catalysis by FtsH and that the additional presence of the transmembrane sequence is required for this enzyme to degrade a membrane protein even under detergent-solubilized conditions.

Escherichia coli FtsH is a membrane-bound metalloprotease which contains an AAA ATPase domain (*1*−*3*). Its substrates include both integral membrane proteins and soluble cytoplasmic proteins (*4*, *5*). FtsH catalyzes processive degradation of membrane proteins, in which the substrate protein is probably dislocated to the cytoplasm where the enzymatic active sites of this enzyme reside (*6*). As many other ATP-dependent proteases, FtsH is a multimeric enzyme (*3*, *7*). Its association with a membrane-bound HflKC complex is also known (*8*). HflKC appears to have a regulatory function against FtsH (*8*, *9*). The N-terminal region of FtsH is important not only for the membrane association but also for the homooligomerization and for the association with HflKC (*3*, *7*, *10*).

To study the significance of multimerization and membrane association of FtsH, we either deleted the N-terminal region totally (FtsH(ΔTM)) or replaced it with the leucine zipper sequence of GCN4 (Zip-FtsH(ΔTM)) or with a transmembrane region from LacY (LacY-FtsH) (*3*). In vivo examinations of these FtsH derivatives showed that FtsH-(ΔTM) had no protease function but it was activated against a cytoplasmic substrate (σ^{32}) by an attachment of the leucine zipper. However, Zip-FtsH(ΔTM) was still inactive against membrane proteins such as SecY. On the other hand, the LacY-FtsH hybrid protein degrades both the cytoplasmic and membrane proteins efficiently. Several lines of evidence suggested that tethering of LacY-FtsH to the membrane induces its oligomerization through intrinsic but weak interaction between the cytoplasmic domain of FtsH. These in vivo results indicated that (i) homooligomerization is crucial for the protease function of FtsH and that (ii) a transmembrane region is required for degradation of membrane proteins.

In the present study, we purified the FtsH derivatives and investigated their ATPase as well as the protease activities in vitro. DMSO[1] was found to stimulate activities of FtsH, possibly by stabilizing its structure, and enabled enzymatic assays of the FtsH derivatives. The results suggest that oligomerization is required for the ATPase activity and that a hydrophobic transmembrane region plays a role in degradation of membrane proteins even in detergent extracts.

MATERIALS AND METHODS

Purification of FtsH. Cells of TY024 (*ompT*::*kan, F′lacI*^{q}) (*7*) carrying pSTD113 (*ftsH-his*$_6$*-myc*) (*7*), pSTD219 (*ftsH-(ΔTM)-his*$_6$*-myc*) (*3*), pSTD348 (*lacY-ftsH-his*$_6$*-myc*) (*3*), or pSTD430 (*zip-ftsH(ΔTM)-his*$_6$*-myc*) (*3*) were grown at 37 °C for 3−6 h in L broth containing 1 mM isopropyl β-D-thiogalactopyranoside, 1 mM cAMP, and 50 μg/mL ampicillin. Membrane and soluble fractions were prepared by

† This work was supported by grants from the Ministry of Education, Science, Sports and Culture, Japan (to Y.A. and K.I.), and from CREST, Japan Science and Technology Corporation (to K.I.).

* To whom correspondence should be addressed. Telephone: 075-751-4040. Fax: 075-771-5699 or 075-761-5626. E-mail: yakiyama@virus.kyoto-u.ac.jp.

[1] Abbreviations: DMSO, dimethyl sulfoxide; NTA, nitrilotriacetic acid; PAGE, polyacrylamide gel electrophoresis.

10.1021/bi010039w CCC: $20.00
Published on Web 06/01/2001

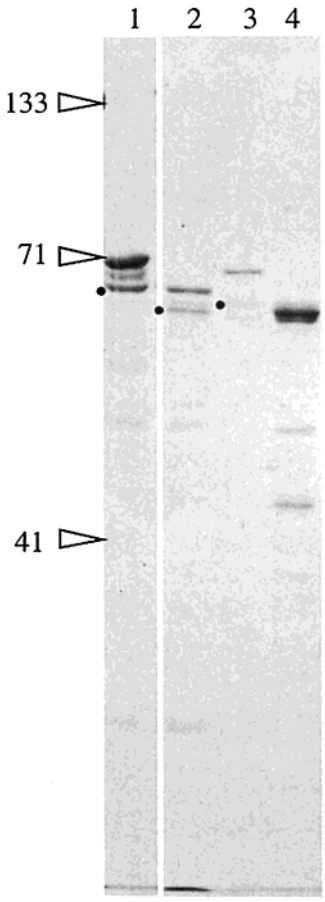

FIGURE 1: Purified FtsH-His_6-Myc and its derivatives. Ni−NTA affinity-purified preparations of FtsH-His_6-Myc (lane 1, 0.8 μg), LacY-FtsH-His_6-Myc (lane 2, 0.4 μg), Zip-FtsH(ΔTM)-His_6-Myc (lane 3, 0.1 μg), and FtsH(ΔTM)-His_6-Myc (lane 4, 0.5 μg) were separated by 10% SDS−PAGE and visualized by Coomassie brilliant blue staining. Positions of molecular mass markers (shown in kDa) are indicated. The dots indicate self-cleaved products of FtsH-His_6-Myc, LacY-FtsH-His_6-Myc, and Zip-FtsH(ΔTM)-His_6-Myc.

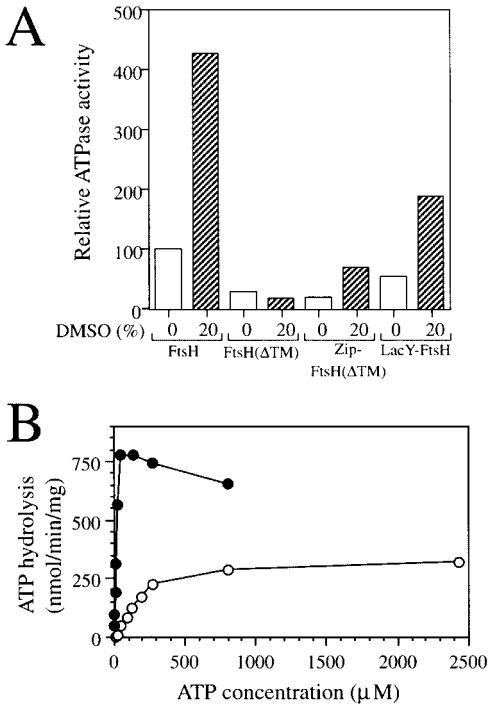

FIGURE 2: ATPase activity of the FtsH derivatives and effects of DMSO on it. (A) ATPase activity of the FtsH derivatives was measured in the presence or the absence of 20% DMSO. The relative values of the ATPase activities to that of FtsH-His_6-Myc in the absence of DMSO are depicted. (B) ATP hydrolysis of FtsH-His_6-Myc at various ATP concentrations was measured in the presence (closed circles) or the absence (open circles) of 20% DMSO.

ultracentrifugation after disruption of cells by a French pressure cell. FtsH-His_6-Myc and LacY-FtsH-His_6-Myc were purified from the membrane fractions, whereas FtsH(ΔTM)-His_6-Myc and Zip-FtsH(ΔTM)-His_6-Myc were purified from the soluble fraction essentially as described previously (*3*, *11*).

ATPase Activity Assay. ATPase activities of the purified proteins were measured by an enzyme-coupled method essentially according to Mori et al. (*12*). The assay mixture (200 μL) typically contained 3 μg of a purified enzyme, 51 mM Tris-HCl, pH 8.1, 5 mM $MgCl_2$, 25 μM zinc acetate, 10 mM 2-mercaptoethanol, 30 mM KCl, 1% glycerol, 0.05% Nonidet P-40, 0.8 mM ATP, 0.25 mM NADH, 2.4 mM phosphoenolpyruvate, 10 units of pyruvate kinase, and 15 units of lactate dehydrogenase with or without 20% DMSO. Oxidation of NADH was monitored at 37 °C by continuous measurement of absorbance at 340 nm.

Proteolytic Activities of FtsH. In the standard assay of resorufin-labeled casein degradation, the purified FtsH derivatives were incubated with the substrate (200 μg/mL) at 37 °C in buffer containing 52.8 mM Tris-HCl, pH 8.1, 5 mM $MgCl_2$, 25 μM zinc acetate, 84.8 mM KCl, 2.8% glycerol, 0.14% Nonidet P-40, 10 mM 2-mercaptoethanol, 5.7 mM imidazole, and 5 mM ATP in the presence or the absence of 20% DMSO. A portion of each sample was withdrawn at intervals and mixed with the same volume of 7% trichloroacetic acid. After 10 min or more at room temperature, samples were centrifuged. The supernatants were mixed with 1.5 volume of 0.5 M Tris-HCl, pH 8.8, and absorbance at 574 nm was measured. For the SecY degradation assay, SecY and the FtsH derivatives were incubated at 37 °C in buffer containing 54 mM Tris-HCl, pH 8.1, 5 mM $MgCl_2$, 25 μM zinc acetate, 118 mM KCl, 3.9% glycerol, 0.22% Nonidet P-40, 10 mM 2-mercaptoethanol, 7.9 mM imidazole, and 5 mM ATP in the presence or the absence of 20% DMSO. A portion of each sample was withdrawn at intervals and mixed with the same volume of 2× SDS sample buffer (*13*). Proteins were separated by 15.1% acrylamide−0.12% *N*,*N*′-methylenebis(acrylamide) gel (*14*) and detected by immunoblotting with anti-SecY serum (*15*). Visualization was done by means of an ECL detection kit (Amersham Pharmacia Biotech) and a Fuji LAS1000 lumino-image analyzer.

Protease Digestion of FtsH. Trypsin digestion of the FtsH derivatives was carried out as described previously (*16*). Briefly, the purified proteins (0.1 mg/mL) were incubated with trypsin (5 μg/mL) at 0 °C for 32 min in buffer containing 50 mM Tris-HCl, pH 8.1, 5 mM $MgCl_2$, 25 μM zinc acetate, and 10 mM 2-mercaptoethanol in the presence or the absence of 5 mM nucleotide, either ATP, ATPγS, or ADP. Samples were mixed with the same volume of 2× SDS sample buffer containing 2 mM phenylmethanesulfonyl fluoride, 6 mM *p*-toluenesulfonyl-L-lysine chloromethyl ketone hydrochloride, and 2 mM Pefablock (Merck) and heated at 37 °C for 5 min. Subtilisin digestion was carried

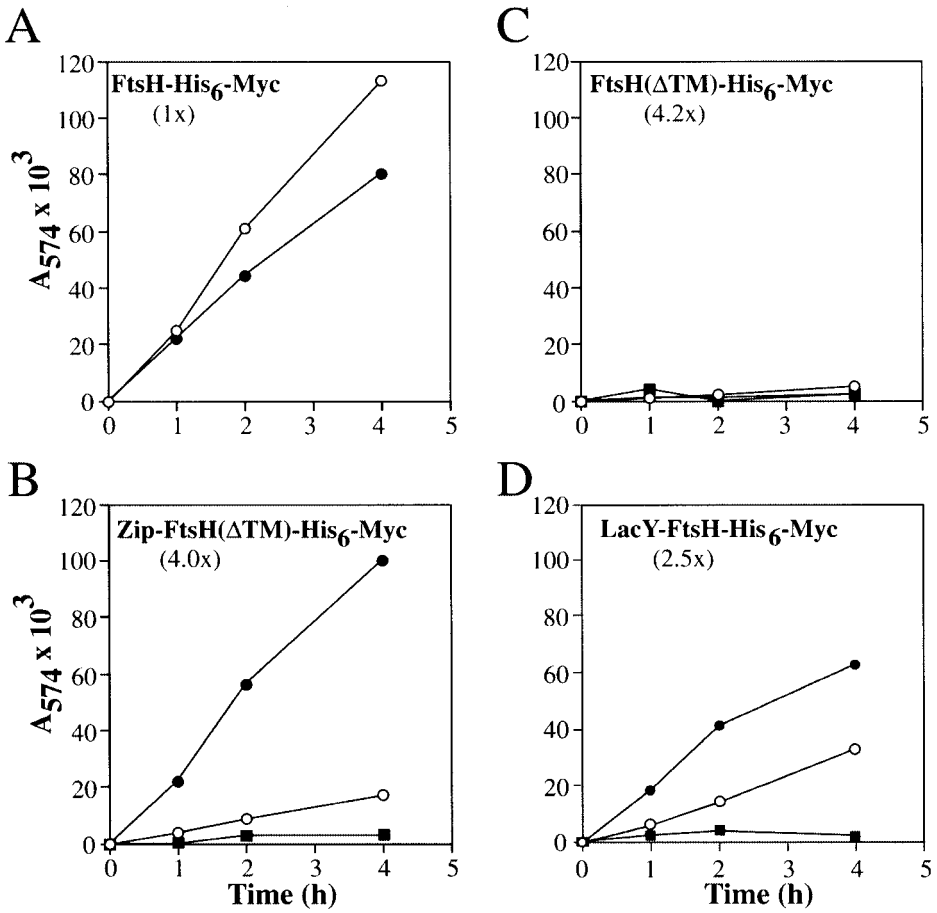

FIGURE 3: Degradation of resorufin-labeled casein by the FtsH derivatives. FtsH-His_6-Myc (20 μg/mL, A), Zip-FtsH(ΔTM)-His_6-Myc (73 μg/mL, B), FtsH(ΔTM)-His_6-Myc (65 μg/mL, C), or LacY-FtsH-His_6-Myc (47 μg/mL, D) was incubated with resorufin-labeled casein (200 μg/mL) at 37 °C in the presence of 5 mM ATP (open circles), 5 mM ATP plus 20% DMSO (closed circles), or 20% DMSO (closed squares). A portion of the samples were withdrawn at the indicated time points, mixed with the same volume of 7% trichloroacetic acid, and centrifuged. Absorbance at 574 nm of the supernatant was measured. Relative molar concentrations of the FtsH derivatives used for the assays are indicated in parentheses.

out similarly in the presence of 5 mM ATP using the purified proteins (0.1 mg/mL) and subtilisin (0.3 μg/mL). Proteins were separated by 10% SDS−PAGE (*13*) and visualized by Coomassie brilliant blue staining.

Denatured PhoA Binding Assay. FtsH-His_6-Myc (1.4 μg) or FtsH(ΔTM)-His_6-Myc (0.96 μg) was incubated with *E. coli* alkaline phosphatase (PhoA; 0.16 μg), which had been denatured by the treatment with 6 M urea and 1 M 2-mercaptoethanol (*17*), at 0 °C for 30 min in buffer containing 50 mM Tris-HCl, pH 8.8, 5 mM $MgCl_2$, 25 μM zinc acetate, 60 mM urea, and 20 mM 2-mercaptoethanol. Then, the sample was applied to a Ni−NTA−agarose spin column, which was washed twice with 200 μL of buffer containing 10 mM Tris-HCl, pH 8.1, 300 mM KCl, 10% glycerol, 0.5% Nonidet P-40, 10% glycerol, 20 mM imidazole, and 10 mM 2-mercaptoethanol, and eluted with the same buffer but containing 250 mM imidazole. Proteins in each fraction were precipitated with 5% trichloroacetic acid and analyzed by 10% SDS−PAGE followed by immunoblotting using anti-FtsH (*8*) and anti-PhoA (5 Prime 3 Prim, Inc.).

RESULTS

ATPase Activities of the FtsH Mutants and Their Stimulation by DMSO. FtsH-His_6-Myc and its derivatives with an altered N-terminal region, FtsH(ΔTM)-His_6-Myc, Zip-FtsH-(ΔTM)-His_6-Myc, and LacY-FtsH-His_6-Myc, were purified by means of Ni−NTA affinity chromatography (Figure 1). Zip-FtsH(ΔTM)-His_6-Myc and LacY-FtsH-His_6-Myc underwent in vivo self-cleavage around the junction between the N-terminally attached sequences and the cytoplasmic domain, as described previously (*3*). Thus, these preparations contained significant fractions of self-cleavage products.

ATPase activities of the FtsH derivatives were much lower than that of the wild-type FtsH-His_6-Myc protein. FtsH-(ΔTM)-His_6-Myc, Zip-FtsH(ΔTM)-His_6-Myc, and LacY-FtsH-His_6-Myc were 30%, 20%, and 55% active, respectively (Figure 2A). We found that addition of DMSO (20%) stimulated the ATPase activities of Zip-FtsH(ΔTM)-His_6-Myc and LacY-FtsH-His_6-Myc about 3.5-fold. The ATPase activity of wild-type FtsH (FtsH-His_6-Myc) was also stimulated by DMSO (Figure 2). On the other hand, DMSO did not activate FtsH(ΔTM)-His_6-Myc at all.

The stimulatory effect of DMSO was concentration-dependent up to about 20% (data not shown), which activated the ATPase activity of FtsH-His_6-Myc 2.5−4-fold (Figure 2). ATPase measurement with varying concentrations of ATP showed that the half-maximum activity was obtained at 20−30 μM ATP in the presence of DMSO (Figure 2B, closed circles), although the exact K_m value could not be determined

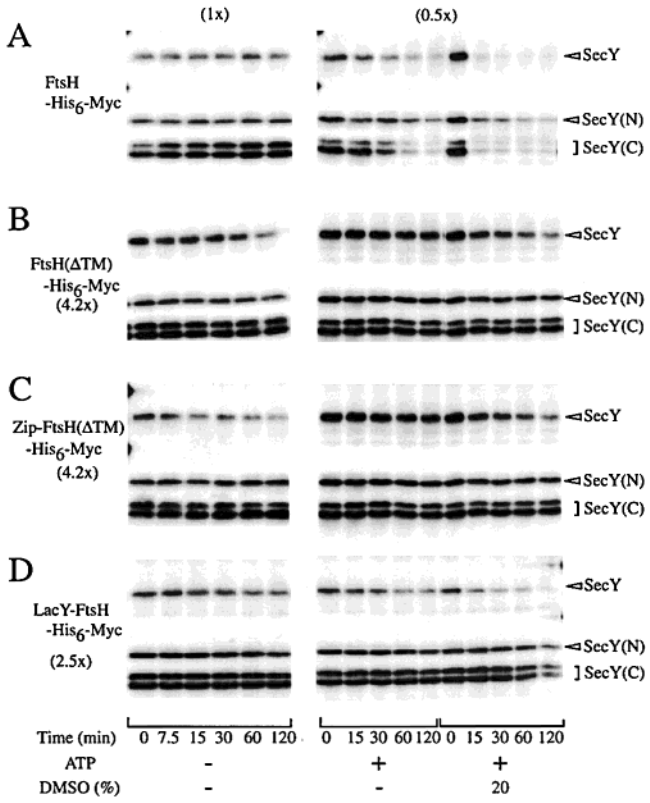

FIGURE 4: Degradation of SecY by the FtsH derivatives. FtsH-His_6-Myc (14 or 28 μg/mL, A), FtsH(ΔTM)-His_6-Myc (95 μg/mL, B), Zip-FtsH(ΔTM)-His_6-Myc (102 μg/mL, C), or LacY-FtsH-His_6-Myc (65 μg/mL, D) was incubated with purified SecY (1 μg/mL) at 37 °C in the presence or the absence of ATP and 20% DMSO. A portion of the samples were withdrawn at the indicated time points and mixed with the same volume of 2× SDS sample buffer. Proteins were separated by SDS−PAGE and analyzed by immunoblotting using anti-SecY antibodies. Relative molar concentrations of the FtsH derivatives used for the assays are indicated in parentheses. SecY, SecY(N), and SecY(C) indicate intact SecY, an N-terminal fragment of SecY, and a C-terminal fragment of SecY, respectively, present in the preparation used in this experiment.

because high ATP concentration somehow inhibited the activity. In the absence of DMSO, the apparent K_m value increased about 10-fold (247 μM) (Figure 2B, open circles).

Proteolytic Activities of the FtsH Derivatives. Our previous in vivo results showed that Zip-FtsH(ΔTM)-His_6-Myc and LacY-FtsH-His_6-Myc were proteolytically active whereas FtsH(ΔTM)-His_6-Myc was not (*3*). We first examined proteolytic activities of the purified FtsH proteins using resorufin-labeled casein as a substrate. In the following assays, 2.5−4-fold more mutant proteins (in molar terms) were used in comparison to wild-type FtsH. As shown in Figure 3, FtsH-His_6-Myc degraded resorufin-labeled casein with almost equal efficiencies in the presence and in the absence of DMSO (Figure 3A). In the absence of DMSO, the casein-degrading activities were very low for all the three mutant proteins (Figure 3B−D, open circles). However, Zip-FtsH(ΔTM)-His_6-Myc and LacY-FtsH-His_6-Myc were markedly activated in the presence of 20% DMSO (Figure 3B,D, closed circles). In contrast, FtsH(ΔTM)-His_6-Myc did not significantly degrade casein even in the presence of DMSO (Figure 3C, closed circles).

We next examined the proteolytic activities of these FtsH derivatives against a membrane-bound substrate, SecY

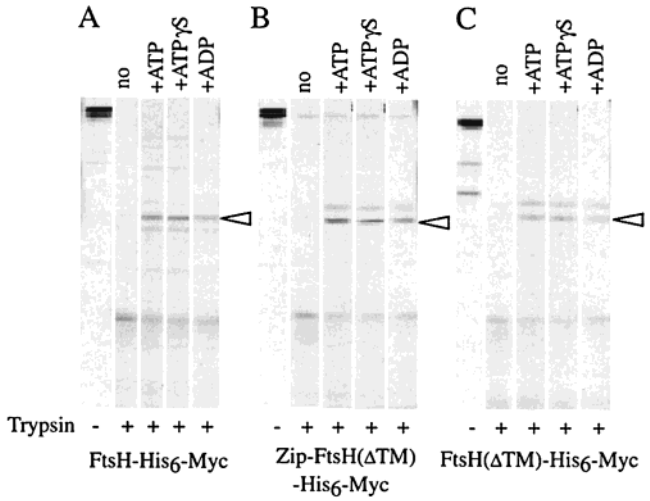

FIGURE 5: Nucleotide-induced conformational changes in the FtsH derivatives. FtsH-His_6-Myc (A), Zip-FtsH(ΔTM)-His_6-Myc (B), and FtsH(ΔTM)-His_6-Myc (C) were incubated with 5 μg/mL trypsin at 0 °C for 32 min in the presence of the indicated nucleotides (5 mM). The reactions were terminated by mixing the samples with SDS sample buffer containing protease inhibitors. Proteins were separated by 10% SDS−PAGE and stained with Coomassie brilliant blue. An arrowhead indicates a trypsin-resistant 33 kDa fragment.

(Figure 4). A purified preparation of SecY was incubated with the FtsH derivatives, and its degradation was examined by immunoblotting. The SecY preparation used contained smaller fragments, SecY(N) and SecY(C), that had been generated presumably by an OmpP-catalyzed cleavage after cell disruption (*18*). We observed a decrease in the intensity of intact SecY even when SecY alone was incubated at 37 °C, especially in the presence of DMSO (data not shown; see also Figure 4). This must have been due to the formation of some SDS-resistant aggregates (*16*). To monitor the true proteolysis, we focused on the decrease in the intensities of SecY(N) and SecY(C), which did not undergo any self-aggregation. In the absence of DMSO, none of the mutant proteins exhibited any significant proteolytic activity against SecY (Figure 4B−D, middle) while wild-type FtsH-His_6-Myc did so significantly (Figure 4A, middle). Although the DMSO was slightly inhibitory on casein degradation by the wild-type enzyme (Figure 3A), it markedly stimulated degradation of SecY by FtsH-His_6-Myc (Figure 4A, right). In the presence of DMSO, LacY-FtsH-His_6-Myc degraded SecY significantly (Figure 4D, right). This degradation was ATP-dependent (data not shown). However, neither Zip-FtsH(ΔTM)-His_6-Myc nor FtsH(ΔTM)-His_6-Myc was active against SecY irrespective of the presence or the absence of DMSO (Figure 4B,C, middle and right).

ATP-Induced Conformational Changes of the FtsH Derivatives. The above results indicate that FtsH(ΔTM)-His_6-Myc is defective both in ATP hydrolysis and in protein degradation. We examined the ability of the FtsH derivatives to interact with ATP. ATP binding induces a conformational change in FtsH-His_6-Myc, which can be demonstrated by the generation of a 33 kDa trypsin-resistant fragment (Figure 5A; *16*, *19*). The 33 kDa fragment was generated also in the presence of a nonhydrolyzable ATP analogue (ATPγS) or ADP (Figure 5A), confirming that hydrolysis of ATP is not required for this conformation change (*16*, *19*). Similar results were obtained both with Zip-FtsH(ΔTM)-His_6-Myc (Figure 5B) and with FtsH(ΔTM)-His_6-Myc (Figure 5C). These

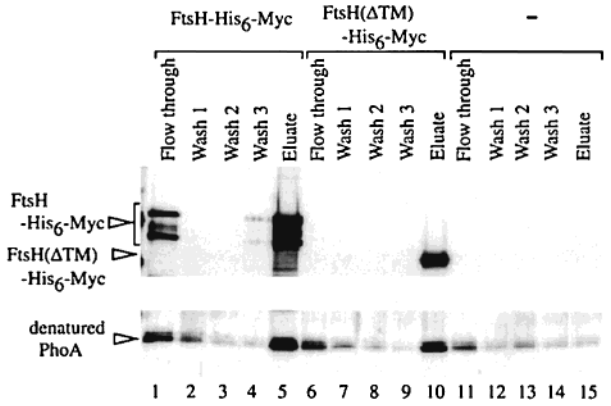

FIGURE 6: Binding of denatured alkaline phosphatase to FtsH-(ΔTM)-His_6-Myc. FtsH-His_6-Myc (1.4 μg) and FtsH(ΔTM)-His_6-Myc (0.96 μg) were mixed with denatured alkaline phosphatase (0.16 μg). The samples were incubated at 0 °C for 30 min and applied to a Ni−NTA−agarose spin column. After being washed three times, bound proteins were eluted with buffer containing imidazole. Flow-through, wash, and eluate fractions were analyzed by SDS−PAGE followed by immunoblotting using anti-PhoA (lower part) and anti-FtsH (upper part) antibodies.

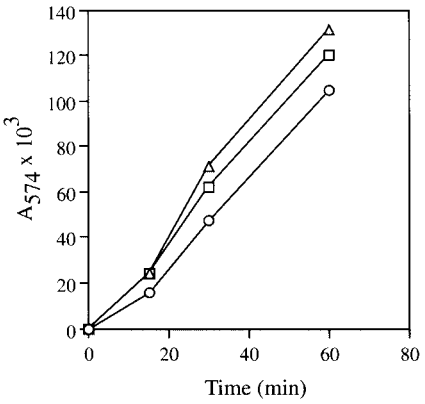

FIGURE 7: Effects of DMSO on subtilisin activity. Resorufin-labeled casein (200 μg/mL) was incubated with 1.5 μg/mL subtilisin at 0 °C in the presence of 0% (circles), 10% (triangles), or 20% (squares) DMSO. Degradation of resorufin-labeled casein was assayed as described in the legend to Figure 3.

results suggest that FtsH(ΔTM)-His_6-Myc can bind ATP, although it cannot hydrolyze the bound ATP.

Polypeptide Binding by the Cytoplasmic Domain. FtsH has an ability to bind a denatured protein in vitro (*16*). We examined whether this ability is preserved for FtsH(ΔTM)-His_6-Myc (Figure 6). FtsH-His_6-Myc or FtsH(ΔTM)-His_6-Myc was mixed with alkaline phosphatase that had been denatured by urea/2-mercaptoethanol treatment. After incubation at 0 °C for 30 min, the mixture was subjected to Ni−NTA affinity isolation. As described previously (*16*), denatured PhoA was coeluted with FtsH-His_6-Myc (lane 5). FtsH(ΔTM)-His_6-Myc also brought down a similar amount of denatured PhoA (lane 10). Without FtsH, essentially no PhoA was recovered in the imidazole eluate (lane 15). The lower recovery of PhoA in the absence of FtsH was possibly due to the formation of aggregates and their nonspecfic retention in the column, which were prevented by interaction with FtsH. Thus, the soluble domain of FtsH retains the denatured protein-binding activity.

DMSO Induces a Conformational Change in FtsH. To address what kind of structural changes might be induced by DMSO, we examined subtilisin susceptibility of FtsH-His_6-Myc, FtsH(ΔTM)-His_6-Myc, and Zip-FtsH(ΔTM)-His_6-Myc. We confirmed that proteolytic activity of subtilisin against resolufin-labeled casein was little affected by 20% DMSO (Figure 7). FtsH-His_6-Myc, FtsH(ΔTM)-His_6-Myc, and Zip-FtsH(ΔTM)-His_6-Myc were treated with subtilisin at 0 °C for various time periods and analyzed by SDS−PAGE (Figure 8). It was found that all of these proteins including monomeric FtsH(ΔTM)-His_6-Myc become much more resistant to subtilisin digestion in the presence of 20% DMSO. These results suggest that DMSO induces some structural changes in the FtsH polypeptide.

DISCUSSION

In this work, we characterized in vitro activities of the FtsH derivatives with an altered N-terminal membrane/oligomerization region, using purified preparations. In the presence of DMSO, Zip-FtsH(ΔTM)-His_6-Myc and LacY-FtsH-His_6-Myc had significant proteolytic activity against casein whereas FtsH(ΔTM)-His_6-Myc did not. The former two, but not the latter, were proteolytically active in vivo (*3*). FtsH(ΔTM)-His_6-Myc was also inactive in the ATPase activity even in the presence of DMSO. Thus, the activities of these proteins correlate with their oligomeric states; Zip-FtsH(ΔTM)-His_6-Myc and LacY-FtsH-His_6-Myc are multimeric and active whereas FtsH(ΔTM)-His_6-Myc is monomeric and inactive. Makino et al. (*19*) also reported that a monomeric MBP-FtsH fusion protein is inactive. We showed that oligomerization is required for ATP hydrolysis by FtsH. Karata et al. (*20*) suggested that the Arg-315 residue in FtsH is involved in hydrolysis of ATP that is bound to the neighboring subunit. Our data support their proposal that an ATPase active site is formed at the subunit interface of the complex. It is remarkable that the ATP-binding ability was observed for the cytoplasmic domain, which is presumably monomeric. Also, FtsH(ΔTM)-His_6-Myc retains the denatured protein-binding activity. Thus, these binding properties may be intrinsic to the individual subunit of this enzyme.

Cross-linking experiments suggested that ATP induces further oligomerization of Zip-FtsH(ΔTM)-His_6-Myc (unpublished experiments). Since similar results were obtained using a nonhydrolyzable ATP analogue (unpublished experiments), the oligomerization does not require hydrolysis of the nucleotides. Our in vivo results that an ATPase-negative cytoplasmic domain of FtsH can interfere with the function of an FtsH derivative (*3*) are consistent with the above observation.

Recent studies show that energy-dependent proteases generally have cylinder-like structures (*21*). Substrates are thought to be sequestered in the cavity for degradation. FtsH also may be in a ring-like structure (*22*). Zip-FtsH(ΔTM)-His_6-Myc might oligomerize, at least in the presence of ATP, to form ATPase active sites as well as a microcompartment for proteolysis.

Zip-FtsH(ΔTM)-His_6-Myc, which was defective in vivo in degradation of membrane-bound substrates such as SecY and YccA (*3*), did not degrade SecY even in detergent extracts. In contrast, LacY-FtsH-His_6-Myc degraded membrane proteins both in vivo (*3*) and in vitro. Given our model (*6*) that FtsH induces dislocation of a membrane protein substrate for the processive degradation, it is conceivable that the membrane localization of FtsH is required for a

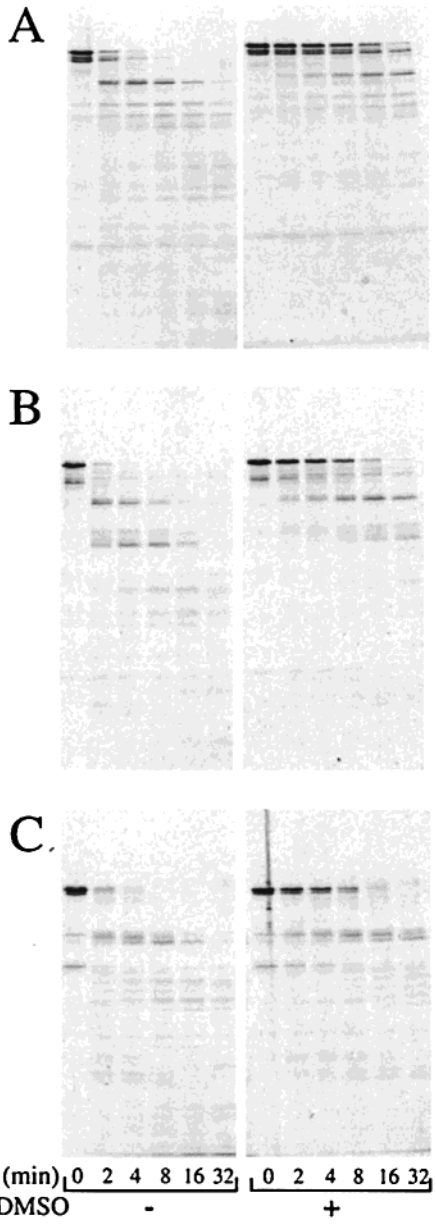

FIGURE 8: Effect of DMSO on subtilisin susceptibility of the FtsH derivatives. FtsH-His_6-Myc (A, 0.1 mg/mL), Zip-FtsH(ΔTM)-His_6-Myc (B, 0.1 mg/mL), and FtsH(ΔTM)-His_6-Myc (C, 0.1 mg/mL) were incubated with 0.3 μg/mL subtilisin at 0 °C for the indicated time in the presence or the absence of 20% DMSO. Samples were analyzed by SDS−PAGE followed by Coomassie brilliant blue staining.

correct positioning of the cytoplasmic domain of FtsH to enable dislocation and proteolysis. The membrane-spanning region of FtsH could contribute to the proper interaction between FtsH and a membrane protein substrate even in detergent micelles, and some dislocation-like movement could even take part in the in vitro reaction. Consistent with these notions, YccA11, a mutant form of YccA which is refractory to FtsH in vivo, was also not degraded by FtsH in detergent extracts (*9*). YccA11 contains only 12 residues at the N-terminal cytoplasmic region, while presence of more than 20 amino acid residues is necessary for the initiation of the FrsH-mediated proteolysis (*23*).

We found that the proteolytic activity of FtsH was significantly stimulated by DMSO. It is possible that DMSO acts on substrates. However, casein degradation by subtilisin (Figure 7), chymotrypsin, or proteinase K (data not shown for the latter two) was not significantly enhanced by DMSO. Instead, DMSO appears to stabilize FtsH, since it increased the subtilisin resistance of FtsH. It also activated FtsH ATPase. We propose that DMSO directly affects the conformation of FtsH. The conformationally activated FtsH may then oligomerize more readily, resulting in the increased catalysis of ATP hydrolysis and in the increased proteolysis of substrates. It was reported that DMSO substantially increases the ATPase activity of the plasma membrane Ca^{2+}-ATPase possibly by facilitating oligomerization of the enzyme (*24*). DMSO-dependent activation may give a clue to our understanding of the enzymatic activities of FtsH.

ACKNOWLEDGMENT

We thank K. Inaba for useful suggestions, H. Mori, S. Chiba, N. Saikawa, K. Kanehara, and N. Shimohata for discussion, and M. Yamada, M. Sano, Y. Shimizu, and K. Mochizuki for technical and secretarial assistance.

REFERENCES

1. Tomoyasu, T., Yuki, T., Morimura, S., Mori, H., Yamanaka, K., Niki, H., Hiraga, S., and Ogura, T. (1993) *J. Bacteriol. 175*, 1344−1351.
2. Tomoyasu, T., Yamanaka, K., Murata, K., Suzaki, T., Bouloc, P., Kato, A., Niki, H., Hiraga, S., and Ogura, T. (1993) *J. Bacteriol. 175*, 1352−1357.
3. Akiyama, Y., and Ito, K. (2000) *EMBO J. 19*, 3888−3895.
4. Akiyama, Y., and Ito, K. (1998) *Handbook of Proteolytic Enzymes* (Barret, A. J., Rawlings, N. D., and Woessner, J. F., Eds.) pp 1502−1504, Academic Press, London, U.K.
5. Langer, T. (2000) *Trends Biochem. Sci. 25*, 247251.
6. Kihara, A., Akiyama, Y., and Ito, K. (1999) *EMBO J. 18*, 2970−2981.
7. Akiyama, Y., Yoshihisa, T., and Ito, K. (1995) *J. Biol. Chem. 270*, 23485−43490.
8. Kihara, A., Akiyama, Y., and Ito, K. (1996) *EMBO J. 15*, 6122−6131.
9. Kihara, A., Akiyama, Y., and Ito, K. (1998) *J. Mol. Biol. 279*, 175−188.
10. Akiyama, Y., Kihara, A., Mori, H., Ogura, T., and Ito, K. (1998) *J. Biol. Chem. 273*, 22326−22333.
11. Akiyama, Y., Kihara, A., Tokuda, H., and Ito, K. (1996) *J. Biol. Chem. 271*, 31196−31201.
12. Mori, H., Sugiyama, H., Yamanaka, M., Sato, K., Tagaya, M., and Mizushima, S. (1998) *J. Biochem. 124*, 122−129.
13. Laemmli, U. K. (1970) *Nature 227*, 680−685.
14. Ito, K. (1984) *Mol. Gen. Genet. 197*, 204−208.
15. Shimoike, T., Taura, T., Kihara, A., Yoshihisa, T., Akiyama, Y., Cannon, K., and Ito, K. (1995) *J. Biol. Chem. 270*, 5519−5526.
16. Akiyama, Y., Ehrmann, M., Kihara, A., and Ito, K. (1998) *Mol. Microbiol. 28*, 803−812.
17. Akiyama, Y., Kamitani, S., Kusukawa, N., and Ito, K. (1992) *J. Biol. Chem. 267*, 22440−22445.
18. Matsuo, E.-i., Sampei, G.-i., Mizobuchi, K., and Ito, K. (1999) *FEBS Lett. 461*, 6−8.
19. Makino, S.-i., Makino, T., Abe, K., Hashimoto, H., Tatsuta, T., Kitagawa, M., Mori, H., Ogura, T., Fujii, T., Fushinobu,

S., Wakagi, T., and Matsuzawa, H. (1999) *FEBS Lett. 460*, 554−558.
20. Karata, K., Inagawa, T., Wilkinson, A. J., Tatsuta, T., and Ogura, T. (1999) *J. Biol. Chem. 274*, 26225−26232.
21. De Mot, R., Nagy, I., Walz, J., and Baumeiste, W. (1999) *Trends Microbiol. 7*, 88−92.
22. Shotland, Y., Koby, S., Teff, D., Mansur, N., Oren, D. A., Tatematsu, K., Tomoyasu, T., Kessel, M., Bukau, B., Ogura, T., and Oppenheim, A. B. (1997) *Mol. Microbiol. 24*, 1303−1310.
23. Chiba, S., Akiyama, Y., Mori, H., Matsuo, E., and Ito, K. (2000) *EMBO Rep. 1*, 47−52.
24. Kosk-Kosica, D., and Roszczynska, G. (1994) *Mol. Cell. Biochem. 140*, 195−199.

BI010039W