Archaeal Proteasomes Effectively Degrade Aggregation-prone Proteins and Reduce Cellular Toxicities in Mammalian Cells

Shin-ichi Yamada, Jun-ichi Niwa, Shinsuke Ishigaki, Miho Takahashi, Takashi Ito, Jun Sone, Manabu Doyu, and Gen Sobue

From the Department of Neurology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya-city, Aichi 466-8550, Japan

The 20 S proteasome is a ubiquitous, barrel-shaped protease complex responsible for most of cellular proteolysis, and its reduced activity is thought to be associated with accumulations of aberrant or misfolded proteins, resulting in a number of neurodegenerative diseases, including amyotrophic lateral sclerosis, spinal and bulbar muscular atrophy, Parkinson disease, and Alzheimer disease. The 20 S proteasomes of archaebacteria (archaea) are structurally simple and proteolytically powerful and thought to be an evolutionary precursor to eukaryotic proteasomes. We successfully reproduced the archaebacterial proteasome in a functional state in mammalian cells, and here we show that the archaebacterial proteasome effectively accelerated species-specific degradation of mutant superoxide dismutase-1 and the mutant polyglutamine tract-extended androgen receptor, causative proteins of familial amyotrophic lateral sclerosis and spinal and bulbar muscular atrophy, respectively, and reduced the cellular toxicities of these mutant proteins. Further, we demonstrate that archaebacterial proteasomes can also degrade other neurodegenerative disease-associated proteins such as α-synuclein and tau. Our study showed that archaebacterial proteasomes can degrade aggregation-prone proteins whose toxic gain of function causes neurodegradation and reduce protein cellular toxicity.

The 20 S proteasome is a ubiquitous, barrel-shaped protease complex responsible for most of cellular proteolysis (1) and is formed by four stacked seven-membered rings (2). The α-type subunits, which are proteolytically inactive (3), form the outer rings, and the β-type subunits, which contain the active site (4), form the inner rings of the complex (5). The 20 S proteasome of archaebacteria (archaea) consists of only one type of each of the α- and β-subunits and is thought to be the evolutionary ancestor of the eukaryotic proteasome (6), which is quite similar in architecture to that of archaea but is composed of seven different α- and seven different β-subunits (6). Archaea do not have the ubiquitin recognition system for protein degradation and are thought to have unidentified tags in its degradation pathway (7). Like eukaryotic cells, archaebacteria also have a regulatory complex for the 20 S proteasome, known as proteosome-activating nucleotidase (PAN) (8). PAN is an evolutionary precursor to the 19 S base in eukaryotic cells and is thought to be necessary for efficient archaebacterial 20 S proteasomal protein degradation (8).

However in vitro, the archaebacterial proteasome has been reported to rapidly degrade polyglutamine aggregates without the help of PAN (9). This PAN-independent degradation by the archaebacterial proteasome inspired us to introduce and test a novel proteolytic facility in mammalian cells. We have chosen the archaebacterium Methanosarcina mazei (Mm) 20 S proteasome, because its optimal growth temperature is around 37 °C, making it suitable to examine its proteasomal effects in mammalian cells.

The eukaryotic ubiquitin-proteasome system degrades aberrant or misfolded proteins that could otherwise form potentially toxic aggregates (10). These aggregate formations in cells are related to the pathogenesis of several common aging-related neurodegenerative diseases, including Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), polyglutamine diseases (e.g. Huntington disease, some spinocerebellar ataxias, and spinal and bulbar muscular atrophy), and Alzheimer disease (AD), which are thought to be associated with the reduced activities of the proteasome (11-15). However, a critical cause of the accumulation of abnormal proteins remains unclear. Solving this common aspect of many neurodegenerative disorders would be a breakthrough in treating these diseases.

In the present study, we show that the Mm proteasome functions in mammalian cells to accelerate the degradation of the following aggregation-prone proteins: mutant superoxide dismutase-1 (SOD1), a causative protein of familial ALS; mutant androgen receptor (AR) with expanded polyglutamine tract, a causative protein of spinal and bulbar muscular atrophy; α-synuclein, an accumulated protein in PD; and tau, an accumulated protein in AD.

* This work was supported by a Center of Excellence grant from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. 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.

1 To whom correspondence should be addressed. Tel.: 81-52-744-2385; Fax: 81-52-744-2384; E-mail: sobueg@med.nagoya-u.ac.jp.

2 The abbreviations used are: PAN, proteosome-activating nucleotidase; SOD1, superoxide dismutase-1; Mm, M. mazei; ALS, amyotrophic lateral sclerosis; AR, androgen receptor; PD, Parkinson disease; AD, Alzheimer disease; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; WT, wild type; NTA, nitrilotriacetic acid; GFP, green fluorescent protein.
Archaeal Proteasomes Degrade Aggregation-prone Proteins

EXPERIMENTAL PROCEDURES

Construction of the Expression Vectors M. mazei Proteasome Subunits α, β, ΔN(2–13)α, and Mutant β (TIC)—The DNA fragment encoding the α-subunit protein (GenBank™ accession number 1480962) was amplified by PCR from the genomic DNA of M. mazei (ATCC) using the following primers: αF (5'-GCGGGTACCCACCATGCAATGCGACCACAGATG) and αR (5'-CGCCCTCGAGTTATTTCTTCTATTCTTTTTGTG). The Δ(2–13) α-subunit (Δα) was amplified using the following primers: ΔαF (5'-GCGGGTACCCACCATGCAATGCGACCACAGATG) and αR. The amplified fragments were inserted into the KpnI and Xhol site of the pcDNA 3.1(+) vector (Invitrogen). The β-subunit (GenBank™ accession number 1479036) was amplified by PCR with the following primers: βF (5'-GCCCTCTAGACCATGCAATTGACAATCATTTA) and βR (5'-GGGACCCGGTTCCTAAAGCTTCTTG) and inserted into the XbaI and AgeI site of the pcDNA3.1(+)/MycHis vector (Invitrogen) to fuse it to a His6 tag. The mutated mβ1-subunit (TIC β-subunit) was generated with a site-directed mutagenesis kit (Stratagene) following the manufacturer's protocol. Construction of pcDNA3.1/MycHis-SOD1 and pcCMV-Tag4-SOD1 vectors (WT, G93A, G85R, H46R, and G37R) (16), pEGFP-N1-SOD1 (WT and G93A) vectors, pCR3.1-AR24Q and pCR3.1-AR97Q vectors, and pcDNA3.1(+) /MycHis-α-synuclein (WT, A53T, and A30P) was described previously (16–18). Six isoforms of tau were amplified by PCR from the pRK172 vectors that were kindly provided by Dr. Michel Goedert and inserted into the Kpnl and Xbal site of the pcDNA3.1 vector (Invitrogen).

Cell Culture, Transfection, and Antibodies—Neuro2a cells and human embryonic kidney 293 (HEK293) cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum. Transfections were performed using Lipofectamine 2000 (Invitrogen) in the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay or Effectene transfection reagent (Qiagen) in other experiments. Antibodies used here were as follows: anti-SOD1 antibody (SOD100; Stressgen Bioreagents), anti-His antibody (Ab-1; Oncogene), anti-α-tubulin antibody (clone B-5-1-1; Sigma), anti-20 S proteasome β-subunit antibody (from Methanosarcina thermophila; Calbiochem), anti-20 S proteasome α-subunit antibody (from M. thermophila; Calbiochem), anti-AR antibody (N-20; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-α-synuclein antibody (LB509; Zymed Laboratories Inc.), and anti-tau antibody (Mouse Tau-1; Chemicon International).

Glycerol Density Gradient Centrifugation—Cells grown on a 10-cm dish were lysed in 1 ml of 0.01 M Tris-EDTA, pH 7.5, by two freeze-thaw cycles, and the lysates were centrifuged for 15 min at 15 000 × g at 4 °C. The cleared supernatants were loaded on the top of a 36-ml linear gradient of glycerol (10–40%) prepared in 25 mM Tris-HCl buffer, pH 7.5, containing 1 mM dithiothreitol and then centrifuged at 80,000 × g for 22 h at 4 °C in a Beckman SW28 rotor (Beckman Coulter Inc.). Following centrifugation, 37 fractions (1.0 ml each) were collected from the top of the tubes with a liquid layer injector fractionator (model number CHD255AA; Advantech) connected to a fraction col-

FIGURE 1. Expression of M. mazei proteasome in mammalian cells. A, schematic illustration of expression vectors used in this study. The deleted sequences of the Δα-subunit are depicted. The TIC β-subunit (mβ1) has three mutated base pairs (a to t, c to g, and a to t). B, Western blot analysis with anti-proteasome α-subunit, anti-proteasome α-subunit, and anti-His antibodies. C, Ni2+ -NTA pull down assay. Pulled down proteins run on SDS-PAGE were probed with anti-proteasome α-subunit. D, chymotrypsin-like activity of the Ni2+ -NTA pulled down samples. This protease activity gradually became higher after transfection. Error bars, S.D. (n = 3). E, glycerol gradient centrifugation experiment. Mm proteasome α- and β-subunits fractionated into nearly the same fractions as did the human 20 S proteasome subunits α1 and α5, αβ- and αβ+, indicating that cells were transfected with mock and Mm proteasome αβ, respectively.
Archaeal Proteasomes Degrade Aggregation-prone Proteins

Neuro2a cells grown on 6-cm dishes and co-transfected with 1 μg of pcDNA3.1/MycHis-SOD1 with mock (0.6 μg), Mm proteasome αβ (0.3 μg each), or Mm proteasome αβ (0.3 μg each). 24 h after transfection, cycloheximide (50 μg/ml) was added to the culture medium, and the cells were harvested at the indicated time points. The samples were subjected to SDS-PAGE and analyzed by Western blotting with anti-SOD1 antibody.

Cycloheximide Chase Analysis—Neuro2a cells grown on 6-cm dishes were transfected with 1 μg of pcDNA3.1/MycHis-SOD1 with mock (0.6 μg), Mm proteasome αβ (0.3 μg each), or Mm proteasome αβ (0.3 μg each). 24 h after transfection, cells were pulse-labeled with [35S]Cys for 60 min and harvested at the indicated time points. After the immunoprecipitation by anti-FLAG antibody (M2; Sigma), the samples were subjected to SDS-PAGE, phosphor-imaged (Typhoon 9410; General Electric Co.), and statistically analyzed by one-way analysis of variance.

Cell Viability Analysis—HEK293 cells were grown on collagen-coated 96-well plates and co-transfected with pcDNA3.1/MycHis-SOD1 (WT, G93A, and G85R) and Mm 20 S proteasome αβ, αβ1, or mock in 12 wells each. The MTS-based cell proliferation assays were performed after 48 h of transfection. Absorbance at 490 nm was measured at 37 °C in a multiple-plate reader (PowerscanHT, Dainippon Pharmaceutical). The assay was carried out in triplicate and statistically analyzed by one-way analysis of variance.

Caspase-3/7 Assay—HEK293 cells were grown on black 96-well plates and co-transfected with pcDNA3.1/MycHis-SOD1 (WT, G93A, and G85R) and Mm 20 S proteasome αβ, αβ1, or mock. 24 h after transfection, the medium was replaced with serum-free medium (Dulbecco’s modified Eagle’s medium). After 24 h, activated caspase-3/7 activity was analyzed by the Apo-ONE homogeneous caspase-3/7 assay (Promega) following the manufacturer’s instructions.

RESULTS

Cloning and Expression of M. mazei Proteasome—We cloned the Mm proteasome α-subunit (GenBankTM accession number 1480962) and β-subunit (GenBankTM accession number 1479036) from genomic DNA of Mm (Fig. 1A) and generated a mutant α-subunit lacking amino acids 2–13, Δ(2–13) α-subunit (Δα) (Fig. 1A). These amino acids (positions 2–13) nor-
mally form a gated channel in the α-ring that regulates substrate entry into the 20 S proteasome (19). We also generated a mutant β-subunit with T1C (mβ1) (Fig. 1A). Thr-1 in the β-subunit of the archaeal proteasome is essential for proteolysis, and Thr-1 mutants lose their proteolytic activities (20). The following experiments were performed in both HEK293 and Neuro2a cells with similar results in both cell lines.

To confirm protein expression of the Mm subunits, HEK293 cells transfected with mock, α, Δα, β, or mβ1 were lysed, subjected to SDS-PAGE, and immunoblotted with anti-proteasome α-subunit, anti-proteasome β-subunit, and anti-His antibodies. Fig. 1B demonstrates that the α- and β-subunit antibodies detected the Mm proteasome α-subunit at 26 kDa, the Δα-subunit around 25 kDa, and the β-subunit at 22 kDa, respectively, and faintly recognized endogenous human proteasome subunits. A Ni²⁺-NTA pull-down assay showed that the Mm proteasome α- and Δα-subunits co-sedimented with the Mm proteasome β- and mβ1-subunits but not with mock (Fig. 1C), and protease activity of the pulled down samples of the cells lysed 48 h after transfection showed significantly higher chymotrypsin-like protease activity in the Mm proteasome αβ than in the αmβ1 or mock-transfected samples (Fig. 1D). This protease activity was confirmed to become gradually higher after transfection (Fig. 1D).

Glycerol density gradient centrifugation fractionated the αβ, Δαβ, and αmβ1 complexes of the Mm proteasome into nearly the same fractions as those of the human 20 S proteasome subunits α1 and α5 (Fig. 1E; data not shown for Δαβ and αmβ1). Moreover, of the anti-His-immunoblotted bands (Fig. 1E), the density of staining in fractions 20–25 accounts for about 80–90% of the total anti-His staining. That these fractions constitute the majority of the anti-α staining as well suggests that about 80–90% of the β-subunit expression is incorporated into the Mm proteasome. These results suggested that the Mm proteasome α1, Δα1, β1, and mβ1-subunits could properly assemble to form four stacked seven-membered rings and that an active Mm proteasome could be reproduced in mammalian cells. The cells expressing Mm proteasome Δαβ displayed cellular toxicity, whereas the cells expressing Mm proteasome αβ showed little toxicity.
**FIGURE 4.** *M. mazei* proteasome reduces the cellular toxicity of mutant SOD1. The dose-dependent rescue effect of Mm proteasome αβ expression on cell viability in SOD1WT- (A), SOD1G93A- (B), and SOD1G85R-transfected HEK293 cells (C) as shown in MTS-based cell proliferation assays. The box plots show the median values (center line of box), the 25th (lower line of box), 75th (upper line of box), 10th (lower T bar), and 90th (upper T bar) percentiles in each group (*n* = 3 × 6 wells). The numbers indicate the dose of DNA transfected in each well of a 96-well plate (µg/well). The expression levels of SOD1, α-subunit, and β-subunit at the analyzed points are shown. D, relative activities of cleaved caspase-3/7 were analyzed with the fluorescent caspase substrate, benzoyloxycarbonyl-DEVD-R110. Production of Mm proteasome αβ prevents activation of caspase-3/7. Positive control value was 3.2 ± 0.2 (S.D.) (*n* = 3 × 4 wells) (1 µM staurosporin, 24 h).
M. mazei Proteasome Degrades Specifically Mutant Superoxide Dismutase-1—We then assessed whether the Mm proteasome actually affects mutant SOD1 protein (SOD1G85R, SOD1G37R, SOD1G93A, and SOD1H46R) expression. In cultured cells, mutant SOD1G85R, SOD1G37R, and SOD1G93A are more likely to form aggregates than is SOD1H46R (16), and cases of familial ALS expressing these mutant forms are also more severe than those expressing SOD1H46R. Western blot analyses demonstrated that the levels of mutant SOD1 were markedly reduced as the expression of Mm proteasome αβ increased (Fig. 2). However, wild-type SOD1 levels were not affected by the expression of Mm proteasome αβ. Furthermore, mutant SOD1 levels were not affected by the expression of Mm proteasome containing the mβ1-subunit in all mutant species, indicating that Mm proteasomal activity was important to reduce the levels of mutant SOD1 proteins. That the expression level of SOD1H46R was less affected by Mm proteasomal expression than other mutant SOD1 species may be associated with the lower toxicity of SOD1H46R.

To determine whether the reduced levels of mutant SOD1 protein were due to accelerated degradation of mutant SOD1 or to the reduction of mutant SOD1 expression, we examined the stability of mutant SOD1 proteins expressed in Neuro2a cells co-expressed with Mm proteasome αβ, mβ1, or mock (Fig. 3, A and B). Chase experiments with cycloheximide, which halts all cellular protein synthesis, demonstrated mutant species-dependent acceleration in SOD1 protein degradation, whereas the expression levels of Mm proteasome α- and β-subunits did not change (Fig. 3A). The degree of wild-type SOD1 degradation was not affected by the expression of Mm proteasome αβ. Pulse-chase experiments further confirmed that 35S-labeled SOD1G93A degradation was significantly accelerated when co-expressed with Mm proteasome αβ but not with Mm proteasome mβ1 or mock (Fig. 3B). These facts strongly suggest that the catalytic center in the Mm proteasome β-subunit is important to accelerate the degradation of mutant SOD1 proteins.

M. mazei Proteasome Reduces Cellular Toxicities of Mutant Superoxide Dismutase-1—Next, we investigated the viability of HEK293 cells evoked by SOD1 (wild-type, SOD1G93A, and SOD1G85R) when co-expressed with Mm proteasome αβ, mβ1, or mock by the MTS-based cell proliferation assay (Fig. 4). We confirmed a linear response between cell number and optical density at 490 nm between 0.85 and 1.30 (data not shown). The viability of cells expressing wild-type SOD1 with Mm proteasome αβ did not change as the transfected DNA doses of SOD1 and Mm proteasome αβ increased (Fig. 4A). However, the viability of cells expressing mutant SOD1 was reduced as the transfected DNA dose of SOD1 increased (Fig. 4, B and C), and this reduction was prevented by the co-transfection with Mm proteasome αβ but not with Mm proteasome mβ1. Toxicities of mutant SOD1 proteins are associated with the activation of caspase family proteins, especially caspase-3 (21). Using fluorescent substrates of activated caspase-3/7 as markers, we analyzed caspase-3/7 activities in the cells co-transfected with SOD1 proteins and with mock, Mm proteasome αβ, and mβ1. Mm proteasome αβ suppressed the activation of caspase-3/7, resulting in reductions of cellular toxicities of SOD1 proteins (Fig. 4D). These results show that Mm proteasome αβ has a protective effect against the decrease in cellular viability evoked by mutant SOD1.

M. mazei Proteasome Co-localizes with Aggregates Formed by Mutant SOD1—In the assembly process of the archaeal proteasome, α-subunit assembly is required for β-subunit incorporation into the proteasome (20), and since the anti-His-stained β-subunit is restricted largely to that incorporated into the Mm proteasome (Fig. 1F), we used anti-His staining to localize the transfected proteasome in Neuro2a cells. GFP-tagged wild-type and G93A mutant SOD1 vectors were transfected along with Mm proteasome αβ into Neuro2a cells, which were then fixed and immunostained with anti-His antibody. Fig. 5A shows that
GFP-positive SOD1\(^{G93A}\) aggregates are also anti-His positive, whereas the cells expressing wild-type SOD1-GFP are diffusely stained with anti-His antibody. There were no GFP-negative inclusion bodies stained with anti-His antibody, indicating that Mm proteasome co-localizes with the inclusion bodies consisting of mutant SOD1 in the vicinity of the nucleus. The percentages of aggregate-positive cells among the GFP-positive cells were determined in Fig. 5B. SOD1\(^{G93A}\) aggregates were significantly reduced when co-expressed with Mm proteasome \(\alpha\beta\).

**M. mazei Proteasome Degrades Specifically Mutant Androgen Receptor with Expanded Polyglutamine Tract and Reduces Its Cellular Toxicity**—To demonstrate the ability of the Mm proteasome to degrade aggregation-prone proteins, we examined the AR with expanded polyglutamine tract (97-repeated glutamine; 97Q) protein, the causative protein of spinal and bulbar muscular atrophy. Similar to the results obtained with SOD1 proteins, Fig. 6A shows that in Neuro2a cells, the levels of mutant AR (97Q) were markedly reduced as the expression of
Mm proteasome αβ increased, but they were unaffected by the expression of the Mm proteasome αmβ1. On the other hand, wild-type AR (24-repeated glutamine; 24Q) levels were not affected by the expression of Mm proteasome αβ. Cycloheximide-chasing analysis demonstrated that the half-life of mutant AR (97Q) was reduced in the presence of the Mm proteasome but not in the presence of the mutant Mm proteasome (Fig. 6B). The viability of cells expressing mutant AR (97Q) was reduced compared with wild-type AR (24Q), and this reduction was attenuated by the co-transfection with Mm proteasome αβ (Fig. 6C). These results show that Mm proteasome αβ can accelerate the degradation of the aggregation-prone mutant AR with expanded polyglutamine tract and possibly protect the cells from its toxicities.

M. mazei Proteasome Degrades Other Aggregation-prone Proteins but Not Non-aggregation-prone Proteins—To determine whether the Mm proteasome degrades other aggregation-prone proteins as well, we examined its effects on α-synuclein (wild-type, A53T, and A30P) and six isoforms of wild-type tau protein in Neuro2a cells. The six tau isoforms contained either three (3L, 3M, and 3S) or four (4L, 4M, and 4S) microtubule binding domains in the C-terminal portion and two (3L, 4L), one (3M, 4M), or no (3S, 4S) inserts of 29 amino acids each in the N-terminal portion. Similar to the results obtained with the mutant SOD1 and AR with an expanded polyglutamine tract, the expression levels of all α-synuclein and tau proteins were reduced in the presence of Mm proteasome αβ (Fig. 7, A and B). Although the degradations of wild-type SOD1 and AR proteins were not accelerated by Mm proteasome, the expression levels of α-synuclein including wild-type and all of the six forms of wild-type tau were reduced.

We also examined whether Mm proteasomes degrade non-aggregation-prone proteins such as GFP or LacZ. Fig. 7C shows that the Mm proteasome does not affect the degradation of the exogenously expressed proteins, GFP and LacZ.

**DISCUSSION**

In this study, we showed that the archaeal Mm proteasome α- and β-subunits properly assembled to have proteolytic activity and accelerate the degradation of aggregation-prone, neurodegeneration-associated proteins in mammalian cells. Archaeal proteasomes contain 14 identical active sites that, although originally classified as chymotrypsin-like, were later shown to cleave after acidic and basic residues (22), and they consist of only one type of each of the α- and β-subunits (6). A comparison between archaeal and eukaryotic proteasomes in vitro showed that archaeal proteasomes are far more active in degrading poly(Q) peptides than are eukaryotic proteasomes (9). We utilized this potential power and manageability of archaeal proteasomes to degrade abnormal proteins that could not be effectively degraded by eukaryotic proteasomes. This is the first report showing that archaeal proteasomes can work to accelerate degradation of aggregation-prone proteins in mammalian cells.

Mm proteasomes promoted degradation of mutant SOD1, AR with an expanded polyglutamine tract, wild-type and mutant α-synuclein, and six isoforms of wild-type tau. The first two proteins, mutant SOD1 and AR with an expanded polyglutamine tract, exhibit toxicity in cell culture models. Mice over-expressing these mutant proteins display abnormal aggrega-
Archaeal Proteasomes Degrade Aggregation-prone Proteins

...tions in their motor neurons and significant loss of motor functions, and they have been used as disease models (23, 24). Mm proteasomes accelerated the degradation of only the mutant forms of these two proteins and not that of the nonaggregating wild-type forms. Furthermore, chasing studies (Fig. 3, A and B) confirmed our belief that Mm proteasomes directly accelerate the degradation of mutant proteins.

However, both the wild-type and two mutants of α-synuclein as well as six isoforms of wild-type tau were also degraded by Mm proteasomes (Fig. 7). α-Synuclein and tau are pathogenically different proteins from SOD1 and AR, since they are known to accumulate as wild-type proteins in the affected lesions of PD and AD, respectively. Aggregation of the presynaptic protein, α-synuclein, has been implicated in synucleinopathies, such as sporadic and familial PD, diffuse Lewy body disease, and multiple-system atrophy (25). In sporadic PD patients, wild-type α-synuclein is accumulated, and increased expression of wild-type α-synuclein is also observed (26). Proteasomal dysfunction has been thought to impair α-synuclein degradation and thereby to facilitate its aggregation (27). Three- and four-repeat wild-type tau are among the proteins characterizedly detected in neurofibrillary tangles formed by paired helical filaments in sporadic AD (28). Decreased proteasomal activity has been also reported in the AD brain (29). α-Synuclein and tau are both relatively easily misfolded, which leads to the formation of aggregates, even in their wild-type forms (30, 31), thus possibly explaining why the Mm proteasomes degraded wild-type α-synuclein and tau. Mm proteasomes might be able to recognize a wide range of aggregation-prone proteins, whereas they do not affect the degradation of correctly folded proteins or misfolded proteins. The gated channel in the archaeal 20 S proteasome has chaperone-like activity to recognize and unfold the aggregation-prone proteins or misfolded proteins. The question raised here is what is the molecular mechanism of such selective, mutant species-dependent degradation. Archaeal 20 S proteasomes contain proteasome-activating nucleotidase, PAN, enabling substrates to enter the proteasomes easily and effectively (8). PAN has a chaperone-like activity to unfold aggregated proteins (32) and is thought to be an evolutionary precursor to the 19 S base in eukaryotic cells (8). Archaeal recognition tags (like ubiquitin tags in eukaryotic cells) have not been identified yet. However, archaeal 20 S proteasomes have been reported to rapidly degrade polyglutamine aggregates in vitro, without the help of PAN (9). Here we confirmed that this PAN-independent degradation by Mm 20 S proteasomes could occur in mammalian cells. Since the pore diameter of the closed gate in 20 S proteasomes is estimated to be much smaller than that of aggregated proteins (33), the question is, how do the unfolded substrate proteins enter the 20 S proteasomes? One hypothesis might be that the α-ring in Mm proteasomes has chaperone-like activity to recognize and unfold the aggregation-prone proteins or misfolded proteins. The gated channel in the α-ring of the archaeal 20 S proteasome is thought to regulate substrate entry into the proteasomes and is assumed to be in either an open (34) or a closed state (2, 33) in vitro. In our experiments, the gate-free Mm 20 S proteasome ∆αβ substantially reduced cell viability, but the Mm proteasome αβ, with the “gate,” had little toxic effect on the cells and, furthermore, accelerated the degradation of mutant proteins. This would be hard to explain if the gate is always in the closed state. There is a possibility that when Mm proteasomes gather, actively or passively, near aggregation-prone proteins, the α-ring opens its gate and unfolds the aggregated proteins, enabling them to enter the proteasomes to be degraded.

Some kinds of molecular chaperones, such as Hsp90, -70, and -27, have been reported to assist in the selective degradation of mutant SOD1 and AR proteins in proteasome degradation pathways (35, 17). However, neither the protein levels of molecular chaperones (Hsp90, -70, -40, and -27) nor the ubiquitylation levels of mutant SOD1 and AR were changed in the presence of Mm proteasome αβ expression (data not shown), thus supporting the idea that endogenous ubiquitin-proteasome degradation pathways possibly did not play an important role in the accelerated degradation of mutant proteins. Further study is needed to elucidate the molecular mechanisms of selective recognition of misfolded aggregation-prone proteins by Mm proteasomes.

In this paper, we demonstrated that Mm proteasomes could effectively degrade neurodegenerative disease-related aggregation-prone proteins in vivo. Further studies are needed to determine whether archaean proteasomes can be available to treat diseases in which toxic gain of proteins is causative.

Acknowledgments—We are grateful to Dr. Keiji Tanaka (Tokyo Metropolitan Institute for Medical Science) and Dr. Peter Zwickl (Max-Planck-Institut fur Biochemie, Abteilung Molekulare Strukturbioalogie) for invaluable support for this study and for preparing the manuscript.

REFERENCES

1. Hershko, A., and Ciechanover, A. (1998) Annu. Rev. Biochem. 67, 425–479
2. Puhler, G., Weinkauf, S., Bachmann, L., Muller, S., Engel, A., Hegerl, R., and Baumeister, W. (1992) EMBO J. 11, 1607–1616
3. Zwickl, P., Kleinz, J., and Baumeister, W. (1994) Nat. Struct. Biol. 1, 765–770
4. Seemuller, E., Lupas, A., Stock, D., Lowe, J., Huber, R., and Baumeister, W. (1995) Science 268, 579–582
5. Grziwa, A., Baumeister, W., Dahmann, B., and Kopp, F. (1991) FEBS Lett. 290, 186–190
6. Baumeister, W., Walz, J., Zuhl, F., and Seemuller, E. (1998) Cell 92, 367–380
7. Zwickl, P., Goldberg, A. L., and Baumeister, W. (2000) Proteasomes: The World of Regulatory Proteolysis, pp. 6–20, Landes Bioscience, Georgetown, TX
8. Zwickl, P., Ng, D., Woo, K. M., Klenk, H. P., and Goldberg, A. L. (1999) J. Biol. Chem. 274, 26008–26014
9. Venkatraman, P., Wetzel, R., Tanaka, M., Nukina, N., and Goldberg, A. L. (2004) Mol. Cell 14, 95–104
10. Ciechanover, A., Orjan, A., and Schwartz, A. L. (2000) J. Cell. Biochem. 77, 40–51
11. Kabashi, E., Agar, I. N., Taylor, D. M., Minotti, S., and Durham, H. D. (2004) J. Neurochem. 89, 1325–1335
12. Bailey, C. K., Andriola, I. F., Kampinga, H. H., and Merry, D. E. (2002) Hum. Mol. Genet. 11, 515–523
13. Chen, Q., Thorpe, J., and Keller, J. N. (2005) J. Biol. Chem. 26, 30009–30017
14. Keck, S., Nitsch, R., Grune, T., and Ullrich, O. (2003) J. Neurochem. 85, 115–122
15. Bence, N. F., Sampat, R. M., and Kopito, R. R. (2001) Science 292, 1552–1555
16. Niwa, J., Ishigaki, S., Hishikawa, N., Yamamoto, M., Doyu, M., Murata, S., Tanaka, K., Taniguchi, N., and Sobue, G. (2002) J. Biol. Chem. 277, 36793–36798
17. Waza, M., Adachi, H., Katsuno, M., Minamimyama, M., Sang, C., Tanaka, F., Inukai, A., Doyu, M., and Sobue, G. (2005) Nat. Med. 11, 1088–1095
18. Ito, T., Niwa, J., Hishikawa, N., Ishigaki, S., Doyu, M., and Sobue, G. (2003) J. Biol. Chem. 278, 29106–29114
19. Benaroudj, N., Zwick, P., Seemu¨ller, E., Baumeister, W., and Goldberg, A. L. (2003) Mol. Cell 11, 69–78
20. Seemu¨ller, E., Lupas, A., and Baumeister, W. (1996) Nature 382, 468–471
21. Sathasivam, S., Grierson, A. J., and Shaw, P. J. (2005) Neuropathol. Appl. Neurobiol. 31, 467–485
22. Dahlmann, B., Kopp, F., Kuehn, L., Hegerl, R., Pfeifer, G., and Baumeister, W. (1991) Biomed. Biochim. Acta 50, 465–469
23. Gurney, M. E., Pu, H., Chiu, A. Y., Dal Canto, M. C., Polchow, C. Y., Alexander, D. D., Caliendo, J., Hentati, A., Kwon, Y. W., Deng, H. X., Chen, W., Zhai, F., Suffit, R. L., and Siddique, T. (1994) Science 264, 1772–1775
24. Adachi, H., Kume, A., Li, M., Nakagomi, Y., Niwa, H., Do, J., Sang, C., Kobayashi, Y., Doyu, M., and Sobue, G. (2001) Hum. Mol. Genet. 10, 1039–1048
25. Trojanowski, J. Q., and Lee, V. M. (2003) Ann. N. Y. Acad. Sci. 991, 107–110
26. Miller, D. W., Hague, S. M., Clarimon, J., Baptista, M., Gwinn-Hardy, K., Cookson, M. R., and Singleton, A. B. (2004) Neurology 62, 1835–1838
27. Liu, C. W., Corboy, M. J., DeMartino, G. N., and Thomas, P. J. (2003) Science 299, 408–411
28. Selkoe, D. J. (1991) Neuron 6, 487–498
29. Keller, J. N., Hanni, K. B., and Markesbery, W. R. (2000) J. Neurochem. 75, 436–439
30. Hashimoto, M., Hsu, L. J., Sisk, A., Xia, Y., Takeda, A., Sundsmo, M., and Masliah, E. (1998) Brain Res. 799, 301–306
31. Khlistunova, I., Biernat, J., Wang, Y., Pickhardt, M., von Bergen, M., Gazova, Z., Mandellkow, E., and Mandelkow, E. M. (2006) J. Biol. Chem. 281, 1205–1214
32. Benaroudj, N., and Goldberg, A. L. (2000) Nat. Cell Biol. 2, 833–839
33. Groll, M., Bajorek, M., Kohler, A., Moroder, L., Rubin, D. M., Huber, R., Glickman, M. H., and Finley, D. (2000) Nat. Struct. Biol. 7, 1062–1067
34. Lowe, I., Stock, D., Jap, B., Zwickl, P., Baumeister, W., and Huber, R. (1995) Science 268, 533–539
35. Patel, Y. J., Payne Smith, M. D., de Belleroche, J., and Latchman, D. S. (2005) Brain Res. Mol. Brain Res. 134, 256–274