Role of the *ompT* Mutation in Stimulated Decrease in Colony-Forming Ability Due to Intracellular Protein Aggregate Formation in *Escherichia coli* Strain BL21

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Recently we found that the cells of *Escherichia coli* strain BL21 producing a fusion protein, GST-Sup35NM, show a much more rapid decrease in colony-forming ability in the stationary phase than control cells. In this study, it was found that an extract of the cells producing GST-Sup35NM forms fibrous protein polymers containing GST-Sup35NM. In the course of the study, we realized that strain BL21 carried the *ompT* mutation. We suspected that the deficiency in OmpT protease was responsible for the observed phenotype. To test this, we introduced the wild-type *ompT* gene into strain BL21, and found that the transformed cells recovered the wild-type phenotype. We concluded that OmpT protease, though known to localize on the cell surface, is involved in protein quality control within the cell.

Key words: yeast prion [PSI⁺]; colony-forming ability of *E. coli*; protein polymer formation; OmpT protease; protein quality control

Prions, mammalian pathogens that cause disorders of the central nervous system, are proteins encoded by the Prm gene. They have at least two distinct conformations. One is pathogenic, and the other is not.1,† The pathogenic form has long been known to construct amyloid-like rods and later was found to be rich in the β-sheet structure in the molecule.2,3) It is now known that there are several proteins that persist in the cell by means of conformational alterations similarly to mammalian prions. Of these, the best known is the [PSI⁺] factor encoded by the *Saccharomyces cerevisiae SUP35* gene.4–6) [PSI⁺] is not only an attractive research object but also a convenient and useful model experimental system of mammalian prions.

The monomeric form of the gene product of *SUP35* is eRF3, a termination factor.7) This protein consists of three functional domains: the C domain (C-terminal 432 amino acids) has eRF3 functions, the N domain (N-terminal 123 amino acids) manifests conformational alteration and polymerization, and the M domain (middle 130 amino acids) appears to act as a linker of the N and C domains.8–10) In the course of studying [PSI⁺], we constructed a gene encoding a fusion protein, GST-Sup35NM, in which Sup35NM was fused to the C-terminus of GST (glutathione S-transferase), and found that *Escherichia coli* strain BL21 producing this fusion protein grew normally to the stationary phase, but decreased in colony-forming ability in the stationary phase much faster than the control cells did.11) We became aware that *E. coli* strain BL21 carried the *ompT* mutation. We guessed that a deficiency of OmpT protease in this strain might be responsible for the observed rapid decrease in colony-forming ability. We carried out experiments to test this speculation. Here we present our results and also describe some additional work which we have done to characterize proteins produced by OmpT protease digestion of GST-Sup35NM.

Materials and Methods

Strains and culture conditions. The *E. coli* strains used in this study are listed in Table 1. LB medium (1% bacto-tryptone, 0.5% yeast extract, and 0.5% NaCl, pH 7.2) was used to grow the *E. coli* strains.12) In case of necessity, LB medium was added with 50 μg/ml

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Abbreviations: Amp, ampicillin; BSA, bovine serum albumin; CBB, Coomassie brilliant blue; CDNB, 1-chloro-2,4-dinitrobenzene; EDTA, ethylene diamine tetra acetic acid; GST, glutathione S-transferase; GSH, glutathione; IPTG, isopropyl-β-D-thiogalactopyranoside; LB medium, Luria-Bertani medium; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate buffer with standard saline; PBST, phosphate buffer with standard saline and Triton X-100; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; TEMED, N,N,N',N'-tetramethylenediamine; Tet, tetracycline
ampicillin (Amp) and/or 20 μg/ml tetracycline (Tet). To stimulate tac promoter, 20 μg/ml isopropyl-β-D-thiogalactopyranoside (IPTG) was added. Cells were cultured at 37°C in a rotary shaker at 120 rpm.

Plasmids. Plasmid pGEX-6P-1 (Amersham Pharmacia Biotech, Tokyo) containing the GST gene of Schistosoma japonicum encoding glutathione S-transferase was used to construct fusion genes. A DNA fragment corresponding to Sup35NM was PCR-amplified using plasmid pYST2 as template, and inserted into the BamHI-XhoI site of plasmid pGEX-6P-1 to obtain pGEX6-NM. The presumed gene product had to include extra eight amino acids (Leu-Glu-Val-Leu-Phe-Gln-Asn-Glu) following the Sup35NM moiety, and seven amino acids (Leu-Glu-Arg-Pro-His-Arg-Gly-Pro) between the GST moiety and the Sup35NM moiety.

Plasmid pOmpT-Tet was constructed by inserting a PCR-amplified DNA fragment containing the wild-type ompT gene (ompT+) into the DraI–DraI site of plasmid pBR322.16 Since the Amp′ gene was partly deleted, pOmp-Tet did not confer the Amp′ phenotype. For simultaneous production of OmpT and Gst-Sup35NM, plasmid pOmpT-Tet was introduced into strain BL21 carrying pGEX6-NM (BL21/pGEX6-NM) to yield BL21/pGEX6-NM/pOmpT-Tet. Strains BL21/pGEX6-NM, BL21/pOmpT-Tet, and BL21/pGEX6-NM/pOmpT-Tet were grown in LB + Amp, LB + Tet, and LB + Amp + Tet media respectively. The GST-SUP35NM gene of pGEX6-NM is under the control of the tac promoter while the ompT+ gene of pOmpT-Tet is under the control of the authentic promoter.

Table 1. E. coli Strains Used in This Study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>BL21</td>
<td>F− dcm gal himB-R (B- mB-lop omt) lon ompT</td>
<td>12</td>
</tr>
<tr>
<td>W3110 M25</td>
<td>F− ara D (lac-proAB) pyrL thi</td>
<td>13</td>
</tr>
<tr>
<td>DH10B</td>
<td>Δ (mrr-hsd RMS-mcrB) mcrA recA</td>
<td>14</td>
</tr>
</tbody>
</table>

Growth curve and colony forming ability. To a 500-ml flask, a 100-ml aliquot of culture medium was added, and the medium was inoculated with 0.3 ml of precultured cells (grown in LB medium for 5 to 10 h). A 1-ml aliquot of the culture was removed at 24-h intervals to measure OD600. At the same time, a 0.2-ml aliquot was removed and spread onto agar LB medium; in case of necessity, an appropriate dilution was made. Colonies appearing on the plate were counted after overnight incubation at 37°C. Throughout the experimental period, 20 μg/ml IPTG was added daily together with 50 μg/ml Amp and/or 20 μg/ml Tet.

DNA manipulation. For DNA manipulation, standard procedures were adopted.12 Restriction enzymes and ligase (Takara, Kyoto, and/or Nippon Gene, Toyama, Japan) were used, as suggested by the manufacturers. PCR was performed using an amplification kit (Toyobo, Tokyo). The reaction mixture (50 μl) contained 10 ng template DNA, 10 pmol sense primer, 10 pmol antisense primer, 4 μl dNTP (2 mM), 5 μl 10× PCR buffer, and 0.5 μl KOD Dash DNA polymerase (2.5 U/μl) (Toyobo). The reaction mixture was overlaid with mineral oil and placed in a Mini cycler™ (M. J. Research, Waltham, MA), and the reaction was carried out for 20 cycles of 96°C for 30 s, 50°C for 15 s, and 74°C for 2 min.

Preparation of cell extracts. Cells were harvested by centrifugation, washed twice with sonication buffer (50 mM Tris–HCl, pH 8.0, 50 mM NaCl, and 1 mM EDTA) and suspended in an equal volume (v/v) of the same buffer. The cell suspension was sonicated with a Microson™ cell disruptor (Heat Systems, Plainview, NY), and a cycle of 10 s sonication and 30 s pause was repeated 10 times. Cell homogenate was centrifuged at 17,400 × g for 10 min to obtain the supernatant, which was used as the cell extract.

Protein assay. Protein was measured by the method of Bradford17 using bovine serum albumin as a control.

Congo red binding assay. Cell extract was adjusted to 0.2 mg protein/ml with buffer C (5 mM potassium phosphate, pH 7.4, and 150 mM NaCl), and incubated at 23°C with gentle shaking (2 strokes per min). Aliquots (0.3 ml) were removed at intervals, mixed with an equal volume of Congo red solution (20 μg Congo red, 5 mM potassium phosphate, pH 7.4, and 150 mM NaCl), and measured for absorption at 540 (A540) and 477 nm (A477). Bound Congo red (CRB) was then estimated by the following equation: CRB = (A540/477) – (A477/46,306).18

Enzyme assay. OmpT protease was assayed by the method of Sugimura and Nishihara.15 Cells were grown in an appropriate medium to mid-logarithmic phase, harvested, washed twice with TE buffer, and suspended in TE buffer. The assay mixture contained 50 mM sodium phosphate buffer, pH 6.0, containing 0.1% TritonX-100 (40 μl), 1 mg/ml dynorphin A (5 μl), and the cell suspension (5 μl). The mixture was incubated at 25°C for 10 min. The reaction was terminated by the addition of 1 N HCl (5 μl). After the cells were removed by centrifugation, the supernatant was analyzed by HPLC using a YMC PROTEIN-RP column (YMC, Kyoto, Japan). OmpT protease activity was defined by μmol dynorphin A cleaved per min under the present assay conditions.

SDS–PAGE and Western blot analysis. An aliquot of the cell extract (100 μg protein) or a 0.5-ml aliquot of each even-numbered fraction obtained by gel-filtration chromatography was subjected to SDS–PAGE using a
10% acrylamide gel (10% acrylamide, 0.3% methylene bis-acrylamide, 0.4% Tris–HCl, pH 8.8, 0.1% SDS, 0.04% APS, and 0.06% TEMED) under a constant current of 20 mA/gel (2 mm/cm² 150 mm). The tank buffer contained 25 mM Tris, 192 mM glycine, and 0.1% SDS. After electrophoresis at 20 mA/gel for 90 min, the gel was stained with CBB by the conventional method.

For Western blot analysis, a gel prepared in the same way as CBB-staining was washed briefly with blotting buffer (100 mM Tris, 192 mM glycine, and 5% methanol) and electro-blotted onto Clear Blot Membrane-p (Atto, Tokyo) using a Horize-Blot apparatus (Atto) at a constant current of 2 mA/cm² for 90 min. The membrane was soaked in blocking buffer (10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 0.2% Triton-X 100, 140 mM NaCl, and 2.7 mM KCl). After gentle shaking for 2 h, 1.5 mg anti-GST antibody (Amersham Pharmacia Biotech, Tokyo) or 5 mg protein equivalent of anti-OmpT protease antiserum was added and allowed to stand for 1 h. The membrane was washed with PBST (10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 0.2% Triton-X 100, 140 mM NaCl, and 2.7 mM KCl) and challenged with a goat anti-rat antibody (1.2 μg) for 1 h. The membrane was washed with PBST and soaked in 8 ml of alkaline phosphatase color-developing buffer (100 mM Tris–HCl, pH 9.5, 100 mM NaCl, and 50 mM MgCl₂) supplemented with 36 μl nitroblue tetrazorium (175 mM in 70% N,N-dimethylformamide) and 28 μl 5-bromo-4-chloro-3-indolyolphosphate p-toluidine (115.3 mM in 100% N,N-dimethylformamide).

Glycerol concentration gradient centrifugation. A 1-ml aliquot of the cell extract containing 50 mg protein was overlaid on a glycerol concentration gradient (5 to 50% v/v, total volume of 10 ml) and centrifuged at 1,600 x g for 1 h. After centrifugation, 1-ml fractions were collected from the bottom of the gradient.

Scanning electron microscopy. A fraction of the highest glycerol concentration was dialyzed overnight against distilled water and lyophilized using a Labconco FREZONE 4.5 (Labconco, Kansas City, MO). The resulting material was placed on a sample stage, and examined with a Keyence VE-8800 scanning electron microscope (Keyence, Tokyo) at an acceleration voltage of 2 kV.

Results
Responsibility of the ompT mutation for the reduced colony forming ability induced by overproduction of GST-Sup35NM

We examined a few E. coli strains in our stock collection for response to production of GST-Sup35NM. The results for representative strains are shown in Fig. 1. Strains BL21 (ompT lon), W3110 M25 (ompT), and DH10B (ompTS- lon+) carrying pGEX6-NM grew practically in the same way to the stationary phase. In the stationary phase, DH10B/pGEX6-NM lost colony-forming ability only gradually, but BL21/pGEX6-NM and W3110 M25/pGEX6-NM lost colony-forming ability much more quickly (Fig. 1, left panel). Next, we transformed the pGEX6-NM-bearing strains with plasmid pOmpT-Tet and found that the colony-forming abilities of BL21/pGEX6-NM and W3110 M25/pGEX6-NM were not entirely different from each other or from that of DH10B/pGEX6-NM or pOmpT-Tet (Fig. 1, right panel). From these results, we concluded that the decreased colony-forming ability in the stationary phase of strains BL21 and W3110 M25 induced by production of GST-Sup35NM was conferred by the ompT mutation, which causes a deficiency in OmpT protease.

Effect of OmpT protease on formation of protein polymers containing GST-Sup35NM

To examine further the action of OmpT protease, we focused our attention on strain BL21, because it was
devoid of Lon protease in addition to OmpT protease; with this strain, we would be able to avoid the complexity associated with Lon protease. As shown in Table 2, strains BL21 and BL21/pGEX6-NM were devoid of OmpT protease activity, whereas strains BL21/pOmpT-Tet and BL21/pGEX6-NM/pOmpT-Tet had OmpT protease activity. The reason strain BL21/pGEX6-NM/pOmpT-Tet had only one-fourth of the OmpT protease of strain BL21/pOmpT-Tet is unknown.

Next we did Congo red binding assay. Strains BL21/pGEX6-NM and BL21/pGEX6-NM/pOmpT-Tet were grown overnight in LB+Amp medium and LB+Amp+Tet medium respectively, and cell extract was prepared from each strain and subjected to Congo red binding assay as described in “Materials and Methods.”

![Congo red binding assay](image1)

**Fig. 2.** The Results of Congo Red Binding Assay. E. coli strains BL21/pGEX6-NM (square) and BL21/pGEX6-NM/pOmpT-Tet (circle) were grown overnight in LB+Amp and LB+Amp+Tet media respectively. Cell extracts were prepared and subjected to Congo red binding assay as described in “Materials and Methods.”

![SDS-PAGE and Western Blot Analysis](image2)

**Fig. 3.** Confirmation for Production of GST-Sup35NM by SDS–PAGE and Western Blot Analysis. E. coli strains BL21 (lane 1), BL21/pGEX6-NM (lane 2), BL21/pOmpT-Tet (lane 3), and BL21/pGEX6-NM/pOmpT-Tet (lane 4) were grown overnight in LB, LB+Amp, LB+Tet, and LB+Amp+Tet media respectively. Cell extract was prepared from each strain and subjected to SDS-PAGE (left panel) or Western blot analysis using an anti-GST antibody (right panel). Lane M gives size markers. Closed and open arrowheads indicate the band present in lane 2 but not in lane 4, and vice versa. The bands marked with closed and open arrowheads correspond to intact and digested GST-sup35NM respectively. For details, see text.

### Table 2. Assay of OmpT Protease Activity

<table>
<thead>
<tr>
<th>Strain</th>
<th>Activity* (unit/ml)</th>
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<tbody>
<tr>
<td>BL21</td>
<td>ND</td>
</tr>
<tr>
<td>BL21/pGEX6-NM</td>
<td>ND</td>
</tr>
<tr>
<td>BL21/pOmpT-Tet</td>
<td>5.18</td>
</tr>
<tr>
<td>BL21/pGEX6-NM/pOmpT-Tet</td>
<td>1.29</td>
</tr>
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</table>

*OmpT protease activity was assayed as described in “Materials and Methods,” and is expressed by the activity unit per volume of cell suspension added to the reaction mixture. The cell suspension was adjusted for OD660 to be 1.0. An average of two independent assays is indicated. ND, not detectable.

was examined by SDS–PAGE (Fig. 3, left panel) and Western blot analysis using an anti-GST antibody (Fig. 3, right panel). The cell extracts of strains BL21 and BL21/OmpT-Tet were used for comparison. It was found that strains BL21/pGEX6-NM (lane 2) or BL21/pGEX6-NM/pOmpT-Tet (lane 4) were grown overnight in LB, LB+Amp, LB+Tet, and LB+Amp+Tet media respectively. Cell extract was prepared from each strain and subjected to SDS-PAGE (left panel) or Western blot analysis using an anti-GST antibody (right panel). Lane M gives size markers. Closed and open arrowheads indicate the band present in lane 2 but not in lane 4, and vice versa. The bands marked with closed and open arrowheads correspond to intact and digested GST-sup35NM respectively. For details, see text.

### Timing of the action of OmpT protease

Next we attempted to examine the action of OmpT protease against GST-Sup35NM in vitro. To this purpose, first we confirmed the production of OmpT protease by strain BL21/pOmpT-Tet. Strain BL21/pOmpT-Tet was grown overnight in LB+Tet medium. The cells were harvested and homogenized by sonication. The cell homogenate was then centrifuged to separate supernatant and precipitate. The separated fractions were subjected to SDS–PAGE (Fig. 4, left panel) and to Western blot analysis using an anti-OmpT protease antiserum (Fig. 4, right panel); strain BL21 was used for

![SDS-PAGE and Western Blot Analysis](image3)

**Fig. 4.** Confirmation of Production of OmpT Protease. E. coli strain BL21/pOmpT-Tet was grown overnight in LB+Tet medium. The cells were harvested and used for SDS-PAGE (left panel) and Western blot analysis using an anti-OmpT protease antiserum (right panel). Lane M gives size markers.
comparison. First it was confirmed that the band pattern was nearly the same between the supernatant fraction and the pellet fraction for the two strains. This was because we intended to obtain a supernatant fraction with high enzyme activity. The conditions of cell homogenization we adopted were very gentle, so that a substantial fraction of the cells remained undisrupted. Next it was confirmed that strain BL21 was almost completely lacking in materials that showed positive signals with anti-OmpT protease antiserum in both the supernatant (lane 1) and the precipitate (lane 2). In contrast, strain BL21/pOmpT-Tet showed a positive signal on a band at a position corresponding to 37 kDa. This band was much more intense in the precipitate (lane 4) than in the supernatant (lane 3). The two less-intense bands present in the precipitate were thought to be degradation products of OmpT protease. This result was in accord with the previous belief that OmpT protease is an outer-membrane protease. At the same time, it was clearly seen that the supernatant contained a substantial amount OmpT protease. To further confirm the intactness of OmpT protease induced by plasmid pOmpT-Tet, we examined OmpT protease activity. As shown in Table 2, strains BL21/pOmpT-Tet and BL21/pGEX6-NM/pOmpT-Tet, but not strains BL21 or BL21/pGEX6-NM, had OmpT protease activity. The reason OmpT protease activity of strain BL21/pGEX6-NM/pOmpT-Tet is about four times less than that of strain BL21/pOmpT-Tet was not clear. We used the supernatant, or cell extract, of BL21/pOmpT-Tet (CE_{BL21/pOmpT-Tet}) as crude OmpT protease in the subsequent experiments.

Then we tested whether OmpT protease would digest the polymerized form of GST-Sup35NM. To this purpose, protein polymers formed in the cell extract of strain BL21/pGEX6-NM were isolated by means of glycerol concentration gradient centrifugation and mixed with CE_{BL21/pOmpT-Tet}. The mixture was incubated, and aliquots removed at intervals were subjected to SDS–PAGE (Fig. 5, left panel) and to Western blot analysis using an anti-GST antibody (Fig. 5, center panel) or an anti-OmpT protease antiserum (Fig. 5, right panel). For comparison, CE_{BL21} was used in place of CE_{BL21/pOmpT-Tet}. It was clearly seen that the band pattern revealed by anti-GST antibody or by anti-OmpT protease remained almost unchanged under up to 48 h of incubation (lanes 5, 6, and 7), indicating that the amounts of GST-Sup35NM (the band marked with an open triangle) and OmpT protease remained unchanged during the experiment. Hence we concluded that the polymerized form of GST-Sup35NM is resistant to digestion with OmpT protease.

The above result led us to the speculation that OmpT protease would digest GST-Sup35NM before its polymerization. To test this, we mixed CE_{BL21/pGEX6-NM} and CE_{BL21/pOmpT-Tet} and incubated the mixture with gentle...
shaking. Then, at intervals, the mixture was subjected to SDS–PAGE (Fig. 6A, left panel) and to Western blot analysis using an anti-GST antibody (Fig. 6A, center panel) or an anti-OmpT protease antiserum (Fig. 6A, right panel); for comparison CE\textsubscript{BL21} was used in place of CE\textsubscript{BL21}/pOmpT-Tet. In this case, the intensity of the 66 kD band (open triangle) decreased with progression of incubation and almost completely disappeared within 3 h. It was further noted that the 47-kDa and 43-kDa bands (arrows a and b respectively) also disappeared with increasing incubation time. On the other hand, the intensity of the 40-kDa band (arrow c) increased and then gradually decreased as incubation proceeded. The intensity of the 38-kDa band (closed triangle) quickly increased in the early stage of incubation and kept increasing, though slowly, up to 48 h. It was also observed that OmpT protease (asterisk) remained constant throughout the duration of the experiment.

Possible cleavage sites of OmpT protease in Sup35NM

From the above results, we concluded that GST-Sup35NM (67 kDa) was digested with OmpT protease to give rise to peptides of 47 kDa, 43 kDa, 40 kDa, and 38 kDa. Of these, the 40-kDa peptide was fairly stable but was further slowly digested to give rise to the 38-kDa peptide, which was highly stable and appeared as the end-product of digestion. Hence it was evident that the monomeric form, unlike the polymeric form, of GST-Sup35NM was susceptible to OmpT protease. It is well-established that OmpT protease specifically cleaves between two consecutive basic residues.\textsuperscript{13,21,22} Taking this into account, we examined the amino acid sequence of Sup35NM and found that it had no arginine (R), and that it had four pairs of lysine (K) in the M domain (Fig. 6B). These KK-pairs localized at positions which coincide well with the sizes of the peptides produced by digestion of GST-Sup35NM (Fig. 6C). It is also known that acidic residue at P2 or P2\textsubscript{0} strongly inhibits the action of OmpT protease.\textsuperscript{21} This confirms our observation that the 38-kDa and 40-kDa bands (the assumed target sequence KK is followed by T and V) are rather intense, whereas the 47-kDa and 43-kDa bands (the assumed target sequence is followed by E) are extremely faint (see Fig. 6A, B).

Appearance of protein aggregates formed after digestion of GST-Sup35NM with OmpT protease

After 3 d of incubation, the reaction mixture of the experiment described above was subjected to fractionation by means of glycerol concentration gradient centrifugation. As shown in Fig. 7, much more protein sedimented to the fraction with the highest glycerol concentration in the experiment added with OmpT protease (panel A) than in the control experiment (panel C). Next we examined the fraction with the highest glycerol concentration of
the experiment with OmpT protease using a scanning electron microscope and observed amorphous entities (Fig. 8A), which were apparently different from the smooth filamentous appearance of protein polymers formed in the cell extract of BL21 producing GST-Sup35NM (Fig. 8B).11)

Discussion

We have reported that E. coli strain BL21 producing a fusion protein, in which the prion-forming domain of S. cerevisiae Sup35 was fused to the C-terminus of glutathione S-transferase, grew normally to the stationary phase and rapidly decreased in colony-forming ability thereafter.11) In this report, we present evidence that this phenotype is lost if the wild-type ompT gene is introduced into strain BL21 producing the fusion protein, GST-Sup35NM. We further found that strain BL21 producing both GST-Sup35NM and OmpT protease did not accumulate GST-Sup35NM. Moreover, it was found that while the polymerized form of GST-Sup35NM was resistant to OmpT protease, the monomeric form of it was not. By comparing the sizes of peptides produced by digestion of GST-Sup35NM with OmpT protease with the amino acid sequence of Sup35NM, we reached the conclusion that OmpT protease cleaves at four and only four KK-pairs present in the M domain of GST-Sup35NM.

OmpT protease, or protease VII, is a well-established outer membrane protease present in E. coli.13,19) It is thought to play a central role in protecting from the entry of foreign agents such as porin toxins into the cell.23,24) It is also known to be involved in the maturation of secreted proteins and in in vivo degradation of foreign proteins.20,25,26) It is also known that ompT is expressed in E. coli producing recombinant proteins,27,28) and that OmpT protease associates with intracellular protein aggregates,29) indicating that it plays a central role in E. coli protein quality control. Mutants lacking this enzyme are constructed and are used as expression hosts for recombinant proteins.30) In
the present study, we show that OmpT protease acts within the cell, and that it acts on the monomeric form of GST-Sup35NM but not on the polymeric form. Moreover, our circumstantial but fairly reliable evidence indicates that OmpT protease cleaved GST-Sup35NM at four and only four KK-pairs in the M domain. Our data support the conclusion that OmpT protease plays a vital role in protein quality control. Our data strongly suggest that Lon protease, an ATP-dependent protease that is induced by heat shock,31–33 is not effective in the degradation of GST-Sup35NM.

Our data also indicate that peptides produced by cleavage of GST-Sup35NM with OmpT protease form amorphous aggregates whose appearance is quite different from the fibrous polymers formed with non-digested GST-Sup35NM. Our results strongly suggest that the amorphous aggregates formed with digested GST-Sup35NM did not impose as detrimental an effect as the fibrous polymers formed with non-digested GST-Sup35NM. Although it is well-established that the N domain alone causes induction of [PSI+] and undergoes conformational alteration,3,8,9) the role of the M domain remains obscure. Possibly it acts as a mere linker (see “Introduction”), but recent work has gradually made it clear that it plays an essential role in manifestation of the structural diversity of [PSI+].10,34–36 Our data suggest that the M domain is indispensable for formation of well-aligned polymers of GST-Sup35NM and support the current view of the function of the M domain.

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References


