Potential Use of Toxic Thermolabile Proteins To Study Protein Quality Control Systems

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SulA is an Escherichia coli division inhibitor with a short half-life whose accumulation results in filamentation. Here, we show that SulA is thermally unstable and forms aggregates at elevated temperatures. This property enables the selection of isolates with mutated protein quality control systems.

Protein quality control systems eliminate damaged or unfolded proteins from the cytoplasm. They comprise both chaperones that refold proteins or rescue them from aggregates and proteases whose function is to degrade those proteins that cannot be repaired by the chaperones (1a). The protein quality control system is essential for maintaining the permissive cellular concentration of regulatory, toxic proteins that have short half-lives. One of these proteins is the division inhibitor SulA, which is responsible for growth inhibition of proteolysis mutants. A previous study of Escherichia coli mutants with defects in all four ATP-dependent proteases (Lon, ClpA, ClpX, and HslVU) indicated that these mutants grow better at 42°C than at 30°C. Growth at 30°C was enabled by introducing a mutation into the sulA gene, which codes for a division inhibitor (7). The suppression of growth inhibition at 42°C was unexpected and unexplained but indicated that at 42°C the effect of SulA is minimized (4, 8–10).

We have previously demonstrated the existence of E. coli proteins that are extremely thermolabile and form aggregates at rather low temperatures (2). Based on these findings and previous indications that SulA associates with DnaJ, which is a co-chaperone in the DnaK chaperone machinery (6), we hypothesized that SulA may also belong to this group of thermolabile proteins. In this case, it would be expected that at 42°C SulA would be inactivated or form aggregates, resulting in reduced levels of active cytosolic protein and the restoration of growth, even that of mutants with defects in all ATP-dependent proteases.

To study the behavior of SulA, we cloned the sulA gene under the control of the pBAD arabinose-inducible promoter (3) and tagged it with the FLAG coding sequence (Sigma) at the C terminus of the gene product (Fig. 1A) to enable monitoring by Western blotting. The SulA-FLAG fusion protein appeared to retain the properties of the wild-type protein. We could show that the SulA protein retained its activity as a division inhibitor even when its carboxy-terminal end was fused to FLAG because the cultures were heavily filamented. In addition, the half-life of the SulA-FLAG fusion protein, about 5 min (Fig. 2), was similar to that previously reported for the SulA protein (6).

Using the SulA-FLAG construct, we showed that at 30°C SulA was soluble and did not appear in the aggregate fraction. However, at 42°C a large fraction of SulA was present in aggregates (Fig. 1B). It should be noted that most E. coli proteins do not form aggregates at these low temperatures (2, 11). These results are consistent with the filamentation phenotype expressed at 30°C but not at 42°C (Fig. 1C). The results support the assumption that the ability of the bacteria to overcome the inhibition of cell division at 42°C is due to the lower effective concentration of SulA in the cytoplasm. Moreover, these results indicate that SulA, in addition to being a proteolytic substrate, is a substrate of the chaperones that rescue proteins from aggregates.

These results demonstrate the complexity of the regulation of the SulA concentration. This concentration is determined not only by proteolysis but also by the activity of a variety of chaperones, including ClpB and the DnaK chaperone complex, that rescue proteins from aggregates. Moreover, the thermolability of SulA indicates thermal regulation of its activity.

On the basis of these findings, we developed a screen for strains with mutated protein quality control systems based on the idea that toxic thermolabile proteins, such as SulA, would inhibit mutants with defective protein quality control and slow their growth at 30°C but not at 42°C. A transposition mutant library was constructed using the pLOF system (4), and the mutant colonies were grown for 7 to 8 h at 30 and 42°C in 96-well plates. The turbidity was monitored, and the ratio of mutant colonies were grown for 7 to8h at 30 and42°C in

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These results demonstrate the potential for using toxic, unstable, and thermolabile E. coli proteins for the study of protein quality control systems and the use of such a protein for the successful screening of strains with mutated systems. Moreover, by using this concept, it is possible to study even mutants with partial blocks in proteolysis and chaperone activity, two components whose interactions and equilibrium are sensed by the well-regulated protein quality control system.

Although several components of the protein quality control system have been well studied previously, it is clear that important functions have not yet been identified in genetic and molecular terms. As an example, the recognition of damaged proteins may well be mediated by the bacterial orthologs of the eukaryotic ubiquitin system. Indeed, one tagging system, specific for Clp substrate proteins synthesized from damaged transcripts, has been described previously (8, 12). Yet, there are no

<table>
<thead>
<tr>
<th>Strain</th>
<th>OD₃₀₀ at:</th>
<th>OD₄₂₀ at 42°C/OD at 30°C</th>
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<tr>
<td></td>
<td>30°C</td>
<td>42°C</td>
</tr>
<tr>
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<td>3.4</td>
</tr>
<tr>
<td>KY2350</td>
<td>1.12</td>
<td>3.22</td>
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<tr>
<td>7141</td>
<td>2.1</td>
<td>3</td>
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*Overnight cultures were diluted 1:100 and grown as described in the legend to Fig. 1. Measurements of the OD₃₀₀ were taken after 7 to 8 h. Transposant 7141 was compared to the wild-type E. coli K-12 strain MG1655 and to its isogenic strain KY2350, in which all cytosolic protease genes have been deleted (ΔclpPX-lon-1196::Cat ΔhslVU1172::Tet) (7).
additional data on the tagging of mature proteins that have been unfolded or damaged. The study of strains with mutated protein quality control systems, as well as those with mutated substrates of this system, like SulA mutant proteins, may help in identifying additional factors involved in protein quality control and lead to a better understanding of the recognition and tagging of systems.

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REFERENCES


5. Reference deleted.


FIG. 3. Proteolysis of HTS. The experiment was performed as described in the legend to Fig. 2 except that the cells carried plasmid pBADmetA (9) and Western blotting was done with rabbit anti-HTS sera as the primary antibody and horseradish peroxidase-conjugated anti-rabbit immunoglobulin G as the secondary antibody. Each point represents an average of results from three independent experiments.