Abstract

Recessive mutations in the slyD (sensitivity to lysis) gene were isolated by selecting for survival after induction of the cloned lysis gene E of bacteriophage pX174 [1]. The slyD− mutation, transduced into the normal pX174 host, Escherichia coli C, confers an absolute block on the plaque-forming ability of the wild-type phage, indicating that slyD is required for E function. slyD encodes a protein with 196 residues. A segment corresponding to the first 142 residues of the predicted SlyD protein has significant similarity throughout its length to the FKBP family of peptidyl-prolyl cis-trans isomerases, or rotamases. The C-terminal 46 codons of slyD encode a remarkable histidine-rich peptide which is a metal-binding domain [2]. This sequence is dispensable for slyD function in E-mediated lysis. Although there is no obvious phenotype associated with the slyD− genotype other than the resistance to E-mediated lysis, overexpression of slyD causes cells to filament and to increase significantly in diameter. Mutations in pX174 can restore the plaque-forming ability of the phage on a slyD− host. These pos (plates on slyD) mutants plate on E. coli C wild-type and slyD−. A model for SlyD involvement in E function and the role of SlyD in the cell is discussed.

Keywords: pX174; Bacterial lysis; FKBP; Metal-binding protein

1. Introduction

pX174 provided the first example of two totally different genes occupying the same DNA [3]. The D and E genes are translated from the same DNA, but in different reading frames. The D gene encodes a scaffolding protein that is required for phage assembly. The E gene encodes a 91-residue protein, the first third of which is hydrophobic and membrane associated. The remainder of the protein is hydrophilic and basic. The E gene has been cloned on expression-controlled plasmids, where induction leads to lysis of the Escherichia coli host [4–6].

2. Lysis of E. coli by pX174

E. coli C is the natural host for bacteriophage pX174. When infected by pX174, the turbidity of an E. coli C culture continues to increase for approximately 30 min. The onset of lysis at this time, with the release of cellular contents, leads to a drop in the turbidity of the culture.

Examination of the infected cells just after the onset of lysis revealed a characteristic septal lesion (Fig. 1), with the membrane-bound cellular contents protruding through the lesion to form a bubble, which rapidly bursts or leaks to release the cellular contents. Induction of the cloned E gene results in a similar phenotype, with the time to lysis and the size of the lesion dependent on growth conditions and the...
level of E gene expression. No endolysin or lysozyme activities are encoded by the phage, so it was hypothesized that there are host genes involved in E-mediated lysis.

3. slyD, a host gene required for E-mediated lysis

In a search for host genes required for E-mediated lysis, Maratea et al. [1] isolated mutants that were resistant to the lethal and lytic effects of the (IPTG-inducible) expression of a cloned E gene. Several independently isolated, spontaneous mutants were mapped to approximately 73 min on the E. coli chromosome. This locus was designated slyD (for sensitivity to lysis). The slyD mutants were recessive to the wild-type slyD. φX174 does not form plaques on E. coli C slyD− [7], nor does it cause lysis of a liquid culture.

Other genes have been suggested to have effects on E-mediated lysis [8–10]. However, in none of these cases has it been demonstrated that E expression is normal. Moreover, the possibility of indirect effects must be considered, since it is already established that E-mediated lysis is associated with continued growth and cell division [4]. Finally, in none of these cases was it shown that the mutation affected cell survival, rather than macroscopic lysis.
4. *slyD* locus cloned and sequenced

The wild-type *slyD* gene was cloned into a *slyD*− host using a linked tetracycline resistance transposon (zhd26::TnlO) at 73 min on the *E. coli* chromosome [7]. A complementation test was used to screen for *slyD* activity. Colonies were cross-streaked against *A172*, a *A* phage in which the normal lysis genes S and R have been deleted and which achieves host lysis by expression of the ϕX174 *E* gene under lacpo control [11]. Lysis indicates *slyD*+, while a lack of lysis indicates *slyD*−.

Complementing clones localized *slyD* to a 963-bp EcoRI−AvaI DNA fragment between *rpsL* and *crp*, at 73.5 min on the *E. coli* chromosome. Sequence analysis revealed three open reading frames: orf72, orf159, and orf196, with orf159 and orf196 in opposite orientations on the same DNA (see Fig. 2). Deletion analysis indicated that either orf159 or orf196 was *slyD*. Deletions in the C-terminal region of orf196 (pSD100AS and pSD100AB in Fig. 2) that remained *slyD*+ favored orf159 as *slyD*. However, cloning the full-length fragment in *Pac* expression vector pJF118 [12] resulted in *slyD*− in either orientation (pSD200EH and pSD200HE), indicating the presence of a cryptic promoter.

In order to determine which open reading frame is *slyD*, nonsense (amber) mutations were put in orf159 or orf196 by site-directed mutagenesis. These amber mutations had little or no effect on the opposite open reading frame. The results were definitive. Constructs with nonsense mutations at codons 10 or 52 in orf159 remained *slyD*+, while those with nonsense mutations at codons 14 or 91 in orf196 became *slyD*−. Furthermore, the nonsense mutations in orf196 were suppressible by *supD*, an amber suppressor. So, orf196 is *slyD*, and the C-terminal portion of SlyD is dispensable for its role in *E*-mediated lysis.

5. *slyD* has a metal binding domain at its COOH-terminus

SlyD was independently and concurrently discovered as WHP (*Wondrous Histidine-rich Protein*), a persistent contaminant in immobilized metal affinity chromatography purification of histidine-tagged het-
erologous proteins in *E. coli* [2]. The protein was found to bind metal ions with a stoichiometry of 1:1, but recent preliminary results suggest multiple metal binding sites (Giedroc and Roof, unpublished).

The last 53 amino acids in SlyD constitute a domain that is rich in histidines (15), cysteines (6), acidic amino acids (5 aspartates and 5 glutamates), and glycines (16). This metal binding domain is dispensable for *slyD* activity (see Fig. 3), indicating that it is the amino-terminal domain that is required for *slyD* activity in E-mediated lysis.

6. *slyD* is an immunophilin (FKBP) homolog

Immunophilins are a class of proteins that bind to immunosuppressing drugs. There are two families of immunophilins: cyclophilins, which bind cyclosporin A, and FKBP, *FK506 Binding Proteins*. Although unrelated in sequence, representatives from both families are peptidyl-prolyl *cis-trans* isomerases, or rotamases. They are involved in protein folding and trafficking, signal transduction pathways, and the modulation of activities [13,14].

The segment containing the first 142 amino acids of *slyD*, the region that is essential for *E*-mediated lysis, shows significant sequence similarity to the FKBP family of rotamases. The prototypical FKBP is the single-domain, cytoplasmic, 12-kDa human FKBP involved in immunosuppression. It is not uncommon for FKBP homologs to have amino-or carboxy-terminal appendages. Examples of FKBP homologs with these attached domains include: MIPs (*macrophage infectivity potentiators*), with amino-terminal do-

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Fig. 4. Overexpression of SlyD. *E. coli* cells containing pSD200EH, shown (A) before induction of *slyD*, and (B) 2.5 h after the induction of *slyD* with IPTG.
mains of approximately 13 kDa [14], a variety of eukaryotic domain fusions [14], and SlyD, with its carboxy-terminal metal binding domain [2,7].

Although distantly related to FKBP, slyD is similar throughout its first 142 residues to E. coli orf149 [15] and P. fluorescens orf150 [16], open reading frames that immediately follow and are in an operon with lsp, the gene encoding signal peptidase II. This relatedness is shown in Fig. 3. slyD, orf149, and orf150 are the only known FKBP homologs that have insertions within the FKBP domain. When the amino acid sequences are aligned with the sequence of the 12-kDa human FKBP, whose structure has been solved [19,20], the two insertions (underlined residues in Fig. 3) are found in the 'unstructured loop' that helps to define the active site pocket of the rotamase [19]. So, these insertions may influence the target specificity of these FKBP homologs. Note that the greatest similarity between slyD and orf149 and orf150 is in one of these insertions (slyD residues 120–127).

7. Overexpression of slyD

When slyD is expressed from an IPTG-inducible tac promoter (pSD200EH), the cells become elongated and wider (see Fig. 4). This change in cell shape is not due to a general effect of inclusion bodies, since SlyD remains soluble and no inclusion bodies are observed. Shortly after removal of the inducer by washing the cells and resuspending in fresh medium, numerous septation events occur (not shown), suggesting that high levels of SlyD may be interfering with cell division and/or cell wall synthesis. This is of interest in light of the septal lesions generated during E-mediated lysis.

8. øX174 mutants that form plaques on a slyD− host

øX174 was plated on a slyD− host. Spontaneous mutants that formed plaques arose at a frequency of approximately 1 in 10^6. These pos mutants are currently being analysed to determine what changes in the phage allow them to form plaques on a slyD− host.

9. Fusions lyse a slyD− host

Translational fusions of the hydrophobic domain of E to lacZ [1,21], to cat [21], or to a sequence of 'random' amino acids [22] result in a gene product that, when induced, lyases its E. coli host. E59ølacZ is an in-frame fusion of the 59-amino-terminal codons of E, which contains the hydrophobic domain, to the eighth codon of lacZ. This translational fusion is expressed normally and produces an active β-galactosidase in slyD+ and slyD− hosts. Surprisingly, the fusion protein is equally lethal and lytic in slyD+ and slyD− hosts [1]. The requirement of slyD for lysis by the other fusions not known. It has been postulated that the C-terminal fusions act as oligomerization domains [1,21], substituting for SlyD in the oligomerization of E. It is also possible that the folding of the E domain is affected in these fusions so that SlyD is no longer required for the generation of the lytic conformation.

10. Conclusion

To date, only two rotamase activities have been reported in E. coli. Both rotamases are from the cyclophilin family. One is found in the cytoplasm and the other in the periplasm [23–25]. Other rotamases may be present but not detectable with the substrate used to find the cyclophilin rotamases. SlyD may act as a rotamase, with E as a substrate, to fold E into its active conformation. Translational fusions of E to other domains could use an alternative folding pathway to lysis which is not possible for wild-type E. This alternative pathway could be spontaneous or could use an FKBP homolog, such as Orf149, to facilitate the folding and/or insertion of the E derivatives into the cytoplasmic membrane.

The overexpression phenotype of SlyD, enlarged and filamented cells with SlyD remaining soluble, suggests a possible relationship between SlyD activity and cell division/cell wall synthesis, with too much SlyD poisoning the normal process. Null mutants demonstrate that slyD is a non-essential gene. In the absence of SlyD, Orf149 or another FKBP homolog could provide the rotamase activity or binding that is required for growth.
A specific interaction between E and SlyD seems likely, with SlyD delivering E to its target, where E would subvert normal cell wall growth and division.

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References