The Superinfection Exclusion Gene (sieA) of Bacteriophage P22: Identification and Overexpression of the Gene and Localization of the Gene Product

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Previous work has shown that the sieA gene of Salmonella bacteriophage P22 is located between the genes mnt and 16. We cloned DNA fragments of the region into multicopy vectors and tested the transformants for mediating superinfection exclusion. Subcloning, phenotypical tests, and DNA sequencing resulted in the identification of the sieA gene. There are two possible initiation codons within one open reading frame of 492 or 480 bp. The deduced amino acid sequence leads to a hypothetical polypeptide with a calculated molecular mass of 18.8 or 18.3 kDa, respectively. According to three hydrophobic regions, all of which are long enough to span the membrane, the product of sieA should be a protein of the inner membrane of a P22-lysogenic cell of Salmonella typhimurium. The SieA protein was moderately overproduced from an expression vector in cultures of Escherichia coli and could be recovered from the membrane fraction.

Superinfection exclusion systems are specified by temperate prophages (e.g., P22, λ, P1, and P2) as well as lytic phages (e.g., T-even phages of Escherichia coli). Early experiments already led to the suggestion that during superinfection exclusion the successful DNA transfer from the phage particle into the cytoplasm of the host cell is inhibited. However, the mechanism by which this inhibition is accomplished was not yet examined in any case. Only recently, studies began in order to identify genes and gene products involved in superinfection exclusion.

In the case of the virulent bacteriophage T4, a superinfection exclusion function is encoded by the imm gene. The Imm protein was shown to be an integral protein (83 amino acids) of the cytoplasmic membrane of the infected E. coli cell (15). Characterization of this protein turned out to be difficult, because overproduction of the protein was lethal for the bacteria (14). The temperate bacteriophage P1 codes for a superimmunity function which was shown to act by superinfection exclusion too (9). Sequencing and overexpression of the sim gene led to the identification of the gene product. The 24-kDa Sim protein is the processed form of the produced polypeptide and is located in the periplasm of the lysogenic cell. This protein could be overproduced and purified (16). Localization of the Imm protein as well as the Sim protein in the bacterial envelope supports the idea that both proteins are involved in inhibition of DNA transfer.

The temperate P22 prophage of Salmonella typhimurium specifies two exclusion systems which interfere with the development of superinfecting phages: the superinfection exclusion systems A and B, represented by genes sieA and sieB, respectively. Both systems have the same specificity concerning exclusion of the heteroimmune Salmonella phages L, MG178, and MG40, but they appear to act independently (32).

The sieB function is encoded in the immC region (33). The sequence of the sieB gene is known (21). It encodes a polypeptide of 22.4 kDa which could be a membrane protein according to its deduced amino acid sequence. This gene has its analog in the lambda genome. Although the two genes of P22 and λ do not share appreciable homology, their deduced amino acid sequences show similar hydrophilicity profiles which might underlie their analogous functions. The mechanism of the sieB function is not yet understood, but there are results indicating that expression of sieB rather leads to abortive infection than exclusion of superinfecting phage (30).

In the case of the sieA gene, no λ-analogous function is known. Superinfecting phages cannot express any functions in sieA-lysogens. Therefore, the favored hypothesis is that the sieA gene product prevents entry of superinfecting phage DNA into the cell. As the superinfecting phage adsorbs to the lysogenic cell, most of the phage DNA should be ejected from the particle, but the DNA does not reach the cytoplasm of the host cell. As the ejected phage DNA is unavailable to exogenous DNase, it is probably released into the periplasm. This release of DNA into the periplasm can be either an abnormal injection process or an intermediate step in the injection process. The A system probably blocks the subsequent transfer of the phage DNA across the inner membrane into the cytoplasm (31). In contrast to the B system, the A system is active not only against superinfecting P22 phages but also against transducing particles (32). That means that it excludes DNA from phage virions regardless of its genetic content.

Susskind et al. (31) mapped the sieA gene by infection experiments with lysogenic strains carrying prophage with deletions directly adjacent to or within the immI region. Interestingly, the immI region of E. coli bacteriophage P1 is organized in a similar manner. The sim gene is encoded within this region, acting by exclusion of superinfecting P1 phage and transducing particles too (9, 16).

The exclusion systems sieA of P22, sim of P1, and imm of T4 seem to be related in terms of their interference with the successful injection of phage DNA into the cell. Therefore, the study of these systems could elucidate new aspects of the mechanism of DNA translocation into the host cell during phage infection.

In this report, we present the DNA sequence of the sieA gene region. Cloning of the gene into an expression vector by PCR led to identification of the gene product. We could show...
that the encoded polypeptide is a protein of the inner membrane of the P22-lysogenic cell.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. The following E. coli strains were used in this study. E. coli C600 F- thi-1 endA1 lacY1 supE44 tonA21 was used for propagation of pBR322 (3) and pF118EH (7) derivatives as well as for the expression of genes from pF118EH derivatives. E. coli XL1-Blue recA1 endA1 gyrA96 thi-1 lacY1 supE44 relA1 (M15 Tn10(Tet')c) was used for transformation with pUC13 (18) and pUC18 derivatives (38).

The wild-type S. typhimurium LT2 strain DB21 (supF, nonlysogenic; 4) obtained from H. Schmieg) was used as host for bacteriophage P22. Wild-type P22 c- and P22 virB-3 (VS K5) were provided by the phage collection of H. Prell (2).

Growth of cells and induction. Cultures of E. coli C600 with pF118EH derivatives were grown at 37°C in tryptone-base yeast (TB) extract supplemented with ampicillin (150 µg/ml) to a titer of 3 x 10^8 to 5 x 10^8 cells per ml. Gene expression from the tac promoter was induced by addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 3 h. Induction of the heat shock genes was achieved by adding absolute ethanol to a final concentration of 4% (13).

Transformation and isolation of plasmid DNA. Preparation and transformation of E. coli cells was performed by the method of Mandel and Higa (17). Plasmid DNA was purified from stationary-phase E. coli cultures by the alkaline lysis procedure of Birnboim and Doly (1). Transformation of S. typhimurium DB21 was done by electroporation using a Bio-Rad Gene Pulser as suggested by O’Callaghan and Charbit (20).

DNA sequencing. The nucleotide sequence of the recombinant plC-plasmid pMR5 being denatured with NaOH was determined with the dideoxy-chain termination method (24) using [α-32P]dATP and the Sequenase 2.0 sequencing kit, purchased from U.S. Biochemical Corp. The entire sequence of the HindIII-PstI DNA fragment of pMR5 was determined for both strands. On the side of the PstI cut, a series of nested deletions was generated with the nested deletion kit (Pharmacia LKB) to allow sequencing of the lower strand. The upper strand was sequenced by using synthetic oligonucleotide primers complementary to sequences of the lower strand.

Cloning of the sicA gene after amplification by PCR. The oligonucleotide primers were synthesized on an Applied Biosystems DNA synthesizer. These were used to amplify and modify the sicA gene with Replitherm (Biozym) and a Perkin-Elmer Cetus thermal cycler. The modified oligonucleotides were as follows (mismatched nucleotides of the PCR primers are underlined, and positions correspond to the DNA sequence in Fig. 2): P-sicA1, 5'GAATTC-GGTCGTGACTCCTGA; P-sicA2, 5'GAATTC-CTGGTGTCGTCACCTGA; P-sicA3, 5'CTCGAG-1169TGCTGCTAG; P-sieA1, 5'GAATTC-673GTGTCTGATTCTCT. The resulting PCR products were inserted into pUC18 by using the SureClone ligation kit (Pharmacia LKB). The sicA genes were then subcloned into the expression vector pF118EH as EcoRI-PstI DNA fragments.

Fractionation of E. coli cells. Fractionation of the cells was performed by treatment of cultures with NaOH by the method of Russel and Model (22). SDS-PAGE. Total protein for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was prepared as described elsewhere (12). Samples of 5 µl were applied onto polyacrylamide slab gels (15%, 1 mm by 10 cm) prepared as described elsewhere (11). Electrophoresis was performed for 3 h at 20 and 40 mA, respectively. Gels were stained with Coomassie brilliant blue G-250 or silver stained by the method of Wray et al. (37). The protein marker mix from Pharmacia (LKB) contained (molecular weights are in parentheses) phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (45,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and α-lactalbumin (14,400).

Neutral sequence accession number. The sequence of the sicA region presented in Fig. 2 was submitted to GenBank. The accession number is L19603.

RESULTS

Mapping of the sicA gene by subcloning experiments. On the physical map of the P22 genome, the sicA gene is located between the mnt gene, which is part of the immI region, and gene J6 (31). In order to localize sicA on a defined DNA restriction fragment, P22 DNA was hydrolyzed with EcoRI. Colonies resulting from shotgun cloning in pBR325 were screened for insertion of the EcoRI DNA fragments B and E. One clone contained the tandem fragment BE in natural orientation (6, 23). The resulting plasmids were named pMR1 (BE), pMR2 (B), and pMR3 (E) (Fig. 1).

Expression of the sicA gene can be easily detected by spotting suitable dilutions of a P22 virB-3 (Xs K5) lysate on lysogenic S. typhimurium cells in comparison with nonlysogenic cells. Lysogenic sicA+ cells effectively exclude the virB-3 P22 phage, although these virulent double mutants cannot be forced into the lysogenic state by the complex immune system because of operator and promoter mutations. Likewise, we tested S. typhimurium DB21 transformants containing the EcoRI DNA fragment B, E, or BE inserted in pBR325 for their SicA phenotypes. Only plasmid pMR1 (bearing the tandem fragment EcoRI BE) confers the SicA phenotype, which
implies that the \textit{sieA} gene should have an \textit{EcoRI} restriction site (Fig. 1). In order to reduce the size of the DNA fragment for DNA sequencing, a P22 \textit{HindIII}-\textit{PstI} DNA fragment (1,750 bp) from \textbf{pMR1} including the \textit{immI} region was cloned into the vector \textbf{pUC13}. The resulting hybrid plasmid \textbf{pMR5} still provides the \textit{SieA} phenotype to \textit{S. typhimurium} (Fig. 1). Deletion of a \textit{HindIII-}\textit{HpaI} and a \textit{HindIII-}\textit{StuI} DNA fragment (5) of \textbf{pMR5} resulted in recombinant plasmids with remaining inserts of about 600 and 400 bp, respectively (Fig. 1).

\textit{S. typhimurium} cells containing one of these plasmids can be infected with virulent phage. It can be deduced from these cloning experiments that the \textit{sieA} gene is located at the 3' end of the \textit{HindIII-}\textit{PstI} fragment and that it contains recognition sites for \textit{HpaI}, \textit{StuI}, and \textit{EcoRI}.

\textbf{Nucleotide sequence of the sieA region.} For the determination of one strand of the \textit{sieA} region, deletion derivatives of plasmid \textbf{pMR5} were used. As there are no suitable recognition sites for further subcloning by restriction endonucleases, the other strand was sequenced mainly with the help of specific oligonucleotides as primers. The sequence presented in Fig. 2 fills the gap between two known sequences. At the 5' end the sequence overlaps 11 bp with the \textit{mnt} region (25), and at the 3' end there is an overlap of 84 bp with the gene \textit{16} region (34).

It was already known from the infections of hybrid plasmid bearing cells that sequences downstream and upstream of the \textit{EcoRI} restriction site between \textit{EcoRI} fragments B and E are essential for expression of the \textit{SieA} phenotype. Within the sequenced \textit{sieA} region, there is one open reading frame including a GTG codon at position 673 and an ATG codon at position 685 (Fig. 2). There are hypothetical promoter sequences in the upstream region with some homologies to typical \textit{E. coli} promoters. The -35 and -10 regions are separated by a recognition site for \textit{HpaI}, which is in good accordance with the negative \textit{SieA} phenotype provided by the plasmid \textbf{pMR5mar} \textit{HindIII} \textit{HpaI}. The sequence AGGAGGT, 4 bp upstream of the hypothetical GTG start codon, is complementary to the 16S rRNA and, thereby, exactly follows the rules for a typical Shine-Dalgarno sequence (26, 28). The hypothetical gene comprises 492 or 480 bp, respectively. The deduced amino acid sequence leads to a hypothetical protein of 18.8 kDa (164 amino acids) or 18.3 kDa (160 amino acids).

The open reading frame has the same orientation as the preceding \textit{mnt} gene, but the two regions probably do not form a transcription unit. A possible termination site of the \textit{mnt} gene is underlined in Fig. 2. Transcription of gene \textit{16}, which is the last gene in the operon of the late genes of P22, is opposed to the identified open reading frame. The stop codons of both
genes are separated by 22 bp (34). In accordance with the phenotypical tests and the location of the EcoRI site within the open reading frame, we identified the 492- or 480-bp open reading frame as the \textit{sieA} gene.

A striking feature on the DNA level is the frequent use of rare codons within the open reading frame. More than 18% of the codons are low-usage codons in \textit{E. coli} (8). On the average, less than 6% of the codons in strongly expressed genes correspond to minor tRNAs. It has been suggested that for genes with a high percentage of low-usage codons an excess of the protein could be detrimental (39). In this respect, the codon choice pattern of the \textit{sieA} gene suggests a low expression level.

The hypothetical protein contains about 49% hydrophobic amino acids. The calculation of the hydropathic index by the method of Kyte and Doolittle (10) implies that the protein could be integral to the cytoplasmic membrane because of three hydrophobic domains, each of which is long enough to span the membrane (Fig. 3A). Like other integral proteins of the inner membrane with more than one membrane-spanning region, the hypothetical SieA protein has no signal sequence for translocation of the amino-terminal domain.

After analysis of the nucleotide sequence data and the deduced amino acid sequence we face at least two problems: on one hand the codon usage might preclude a high expression level of \textit{sieA}, and on the other hand the hypothetical membrane protein character of the gene product could disturb the integrity of the cell envelope in case of overproduction.

**Determination of the translational start of \textit{sieA}**. The open reading frame identified as the \textit{sieA} gene possesses two possible start codons: a GUG codon, located at an optimal distance to the preceding ribosome binding site, and an AUG codon, only four codons downstream. From analysis of the nucleotide sequence in this region, it cannot be clearly decided whether the GUG, the AUG, or both codons are used as start codons in vivo. As our main interest lies in the isolation of the biologically active protein, it was necessary to determine the translational start used in vivo. For this purpose, the 480-bp open reading frame starting at the AUG codon (\textit{sieA}1) and the 492-bp open reading frame starting with the GUG codon (\textit{sieA}2) were amplified by PCR. The choice of the various primers allowed the following modifications of the genes: (i) creation of an EcoRI recognition site directly in front of the respective start codon, (ii) the change of the AUG codon in \textit{sieA}2 to CUG to eliminate the possibility of translation initiation at the second start codon, (iii) elimination of the EcoRI restriction site in the 3′ region without changing the encoded amino acid sequence, and (iv) creation of a PstI restriction site directly downstream of the 3′ end of the gene.

The PCR products \textit{sieA}1 and \textit{sieA}2 were inserted as EcoRI-PstI fragments into the expression vector pJF118EH. Transformants of strain DB21 carrying one of these plasmids (pJF-sieA1 or pJFsieA2) showed the phenomenon of superinfection exclusion. The plating efficiencies of the virulent P22 mutant phage on these different clones were comparable to those of the SieA” transformants presented in Fig. 1. These results show that the encoded polypeptides of 160 and 164 amino acids do not differ significantly in biological activity. Therefore, they both should be able to insert themselves into the cytoplasmic membrane, independently of the length of the N-terminal region preceding the first hydrophobic domain. As both modified genes mediate superinfection exclusion, we suppose that the translational start is the GUG codon on account of its optimal distance (7 nucleotides) to the preceding Shine-Dalgarno sequence. On the other hand, \textit{sieA} expression would not be negatively affected if translation begins 12 nucleotides downstream, at the AUG codon.

**Identification of the \textit{sieA} gene product in \textit{E. coli}**. After integration of the genes \textit{sieA}1 and \textit{sieA}2 into the expression vector pJF118EH, the requirements for effective gene expression should be met. This vector provides the inducible \textit{lac} promoter, the \textit{lacI} gene, and an effective Shine-Dalgarno sequence (11 and 10 bp, respectively) upstream of the inserted \textit{sieA} genes. \textit{E. coli} C600 was transformed with pJF118EH, the requirements for effective gene expression were obviously sufficient for providing the SieA phenotype.

The small amount of SieA protein within the induced \textit{E. coli} cells can have diverse causes, which might coincide or interfere with each other.

(i) According to the deduced amino acid sequence, the hypothetical product of the \textit{sieA} gene is an integral protein of the cytoplasmic membrane of \textit{S. typhimurium}. Membrane proteins are often poorly produced and can disturb the integrity of the bacterial envelope if synthesized at higher rates (14). On induction of \textit{sieA} expression, the growth rate of the culture is reduced, but lysis effects could not be observed.

(ii) The hypothetical protein might be folded properly only if it is inserted into the membrane. Assuming that the SieA protein prevents DNA transfer across the inner membrane by directly or indirectly interacting with specific protein complexes, it might be able to insert itself or associate with the membrane only at specific sites. An excess of protein, remaining in the cytoplasm in an unfolded, prefolded, or incorrectly folded state, probably is exposed to degradation by proteases.

![Hydropathic plot of the hypothetical SieA protein constructed by the method of Kyte and Doolittle (10).](http://jb.asm.org/Downloaded from http://jb.asm.org/)
However, induction of \textit{sieA} expression in different protease mutant strains of \textit{E. coli} (lon, ompT, or degP) was not successful (data not shown). Nevertheless, the instability of the SieA protein can still be one of the causes for the difficulties in detecting the gene product.

(iii) The unfavorable codon usage might cause a limitation in the level of \textit{sieA} expression under induced conditions (27). In \textit{sieA}2, 30 of 164 codons are low-usage codons in \textit{E. coli}; six of them are the least-used codon AGG or AGA. The influence of the minor tRNAs corresponding to the arginine codons AGA and AGG was examined by transforming \textit{E. coli} C600 pJFsieA1 and C600 pJFsieA2 with pUBS520 and pSB101 (kindly supplied by R. Mattes) carrying either the \textit{argU} gene (\textit{dnaY}) (recognizing AGA and AGG) or the \textit{argW} gene (recognizing AGG), respectively, on the compatible plasmid pACYC177. The expression patterns of the C600 double transformants analyzed by SDS-PAGE showed no positive influence of the \textit{argU} or \textit{argW} gene on \textit{sieA} expression. However, these results do not exclude the possibility that \textit{sieA} expression is limited by the occurrence of rare codons besides the AGG and AGA codons, and there still can be a coincidence of different unfavorable causes.

\textbf{Induction of \textit{sieA} under heat shock conditions.} As a membrane protein, SieA might need a hydrophobic surrounding or special partners for achieving its native conformation. A number of proteins are known to achieve their native conformation only in the presence of certain proteins, called chaperone proteins or chaperonines. This class of proteins ensures that polypeptides will either fold or be transported properly. Among the heat shock proteins of \textit{E. coli} are chaperone proteins, e.g., the products of the genes \textit{dnaK}, \textit{dnaJ}, \textit{groEL}, and \textit{groES}.

The heat shock genes of \textit{E. coli} can be induced by incubating growing cultures with ethanol (13). Cultures of \textit{E. coli} C600 (pJFsieA1) and C600(pJFsieA2) were grown to mid-log phase at 37°C. Gene expression from the \textit{tac} promoter was induced by addition of 1 mM IPTG for 3 h. Simultaneously, the heat shock genes were induced by addition of 4% ethanol. Total protein samples of uninduced and induced cultures were analyzed by SDS-PAGE (Fig. 4). In the presence of ethanol during induction of cultures C600 (pJFsieA1) and C600(pJFsieA2), a 19-kDa protein was moderately overproduced. Under these heat shock conditions, the identification of SieA protein is probably not based on increased synthesis of the gene product but, rather, on a greater stability provided by chaperonines.

\textbf{Localization of SieA protein in \textit{E. coli} cells by NaOH fractionation.} By treating \textit{E. coli} cultures with 0.1 N NaOH and subsequent centrifugation, a subset of membrane proteins can be separated from the cytoplasmic and periplasmic proteins. Proteins of the cytoplasmic membrane and the outer membrane are quantitatively partitioned into the NaOH pellet, while the supernatant contains the cytoplasmic and periplasmic proteins (22). Samples from uninduced and induced cultures of \textit{E. coli} C600(pJFsieA1) and C600(pJFsieA2) were taken for total protein analysis and for cell fractionation with NaOH (Fig. 5). After induction of \textit{sieA} expression, the SieA protein is detectable in samples of total protein analyzed by SDS-PAGE and subsequent silver staining. The NaOH fractionation of the culture leads to unequivocal detection of the gene product in the insoluble (membrane) fraction. Within the soluble fraction, the protein is not detectable. The \textit{sieA} gene product, therefore, is most probably a component of the inner membrane, as was concluded from the hydrophobic plot.

Likewise, we investigated the localization of SieA protein synthesized in IPTG-induced cultures of C600(pJFsieA2) under heat shock conditions. (For unknown reasons, the SieA protein deriving from ethanol-treated cells could not be stained by silver. Therefore, proteins of ethanol-treated cells were analyzed by SDS-PAGE stained with Coomassie blue.) An intense signal in the NaOH-soluble (cytoplasmic-periplasmic) fraction of the ethanol-treated culture (Fig. 6, lane 8) supports the hypothesis that upon induction of the heat shock response the SieA protein might be stabilized by chaperone proteins within the cytoplasm. As a consequence, the degra-
FIG. 6. Localization of SieA protein in E. coli produced under heat shock conditions (SDS-17.5% PAGE, Coomassie staining). Induction of gene expression in C600(pJF1) with 1 mM IPTG in the absence and presence of ethanol was performed as for Fig. 5. Lanes 1 to 3, samples of total protein from unduced cells, induced cells, and induced cells treated with 4% ethanol, respectively; lane 4, marker proteins (phosphorylase b [94 kDa], bovine serum albumin [67 kDa], ovalbumin [43 kDa], carbonic anhydrase [30 kDa], soybean trypsin inhibitor [20.1 kDa], and a-lactalbumin [14.4 kDa]); lanes 5 and 6, membrane fractions from cells induced with or without 4% ethanol, respectively; lanes 7 and 8, cytoplasmic-periplasmic fractions from cells induced with or without 4% ethanol, respectively.

Discussion

In the present study, we determined the sequence of the sieA gene and successfully showed that it encodes a cytoplasmic membrane protein. The unfavorable codon usage of the gene as well as the membrane protein character of the gene product might limit the amount of protein under conditions of induced gene expression.

Following the definition of rare codons by Grosjean and Fears (8), the sieA gene contains more than 18% (30 from 164) low-usage codons corresponding to minor rRNAs. Six of them are the least-used codon AGG or AGA. Therefore, the codon choice pattern of the sieA gene suggests a low expression level (8, 35). In addition, the instability of the SieA protein contributes to the difficulties proving the protein in the minicell experiments as well as in Coomassie-stained acrylamide gels of protein from cells grown under physiological conditions. The increase in the amount of SieA protein under heat shock conditions indicates that the stable species of the protein might be located in the inner membrane while those molecules being produced in surplus cannot integrate into the membrane and are usually degraded in the cytoplasm. The chaperone proteins might exert their protective function either by folding the protein molecules correctly or by keeping them in an unfolded state.

The sieA sequences on the DNA and on the amino acid levels show no expanded homologies to other genes or proteins, although there are some very short stretches of homology to glucose transporter proteins and sodium-channel proteins of eukaryotes. However, except for the membrane-spanning regions, there are no motifs indicating a specific property of the gene product. One of the favored hypotheses best explaining the results of sieA exclusion says that the superinfecting phage DNA is excluded at the cytoplasmic membrane of the lysogen (30). Our results support this hypothesis: we could show that the product of the sieA gene is a membrane protein, according to the deduced amino acid sequence and to localization by NaOH fractionation.

The following hypotheses describe some imaginable mechanisms by which the superinfection exclusion system A could exert its function.

(i) The sieA gene product directly interacts with the superinfecting DNA. In 1971, however, Susskind and coworkers already showed that it probably does not act by a DNA modification or restriction mechanism (32).

(ii) The SieA protein might interact with proteins of the superinfecting P22 particle, i.e., with the injection proteins or a hypothetical pilot protein. Interestingly, the infection process of P22 mutants in one of the injection genes (7, 20, and 16) is comparable to the normal process of wild-type phage infecting P22 sieA+ lysogens of S. typhimurium. The phage normally adsorbs to the cell surface, the phage DNA is ejected from the virion, but the DNA does not enter the cytoplasm of the bacterium (30). As the injection proteins probably enter the cell together with the DNA (30), they could be a target for SieA. This prophage-encoded protein could modify or mask the injection proteins of the superinfecting phage and thereby prevent it from recognizing its unknown target sites. The latter might be represented by proteins of the inner membrane responsible for transfer of the phage DNA into the cytoplasm. Supposing that one of the injection proteins or another unknown protein of the P22 virion is a pilot protein, the sieA gene product could remove the protective protein from the superinfecting DNA. This type of mechanism is one of the postulations concerning the immunity function of bacteriophage T4 (19). Here, the imm gene product is supposed to remove the pilot protein (gp 2) of the superinfecting DNA, thereby exposing the DNA to degradation of the host RecBCD enzyme.

(iii) As not only the mechanism of DNA transfer but also the components of such a hypothetical system in S. typhimurium are unknown, one can only speculate about a hypothetical interaction of SieA with a DNA import system or a DNA pore in the cytoplasmic membrane. As the entry of DNA into the cytoplasm of sieA+ lysogens is blocked, we favor the hypothesis that SieA directly or indirectly interacts with components of the hypothetical DNA transfer system located in the inner membrane and/or the periplasm. One could suppose that this prophage-specified membrane protein either alters, removes, or masks a component of the transfer system or displaces a component of the system by representing an analogous but nonfunctional molecule of the system.

Interestingly, we could find an amazing similarity in the hydrophilicity profiles of SieA and TolQ. Although the products of genes sieA and tolQ differ remarkably in size (164 and 230 amino acids, respectively [29]), both proteins have three hydrophobic regions which are distributed along the polypeptide chain in similar manners (Fig. 3). As an integral membrane component of the Tol import system (36), TolQ is involved in the import of group A colicins as well as the DNA of filamentous bacteriophages (29). Although the E. coli protein TolQ is involved in DNA uptake whereas the P22 prophage-specified SieA protein of S. typhimurium is involved in preventing DNA uptake, they both participate in or interact with a DNA transfer system. Whether the striking similarity in hydrophilicity profile is significant for the function of these two proteins needs to be further investigated.

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