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Cloning and Expression in *Escherichia coli* K-12 of the Genes for Major Outer Membrane Protein OmpA from *Shigella dysenteriae*, *Enterobacter aerogenes*, and *Serratia marcescens*

STEWART T. COLE,* INGEBORG SONNTAG, AND ULF HENNING

Max-Planck-Institut für Biologie, D-7400 Tübingen, West Germany

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The outer membranes of many gram-negative bacteria contain a major heat-modifiable protein which shows serological cross-reactivity with the OmpA protein of *Escherichia coli* K-12. Using the cloned gene for the *E. coli* K-12 protein as a DNA-DNA hybridization probe, we were able to identify the corresponding genes from *Shigella dysenteriae*, *Enterobacter aerogenes*, and *Serratia marcescens*. These were cloned in a phage λ vector, and their expression in *E. coli* K-12 was studied. All three OmpA proteins were fully produced and correctly exported to the outer membrane. In several cases, complete or partial restoration of known functions of the *E. coli* K-12 protein was observed.

The OmpA protein is one of the few abundant polypeptides of the outer membrane of *Escherichia coli* K-12 (for a recent review, see reference 36). The 35,000-dalton protein (13) spans the membrane (17) and has several functions: it serves as a mediator in F-dependent conjugation (40, 42, 45) and in combination with the lipoprotein (9) is required for the structural integrity of the outer membrane and the generation of normal cell shape (43). Non-physiologically it serves as a receptor for certain phages (15, 45) and is required for the action of a colicin (12).

The corresponding protein has been identified in a number of other gram-negative organisms, and for several species an immunological cross-reaction with the *E. coli* K-12 protein has been demonstrated (7). This group includes the OmpA protein from *Serratia marcescens*, one of the *Enterobacteriaceae* most distantly related to *E. coli* (11, 35). The *E. coli* K-12 gene, *ompA*, has been cloned, and its nucleotide sequence has been determined (5, 24, 32, 33). As expected from the serological behavior, it was possible, by DNA-DNA hybridization, to identify the corresponding genes from other *Enterobacteriaceae*, and here we report on the cloning of three such genes and their expression in *E. coli* K-12.

MATERIALS AND METHODS

Bacterial strains, phages, and colicin. The strains of bacteria used are listed in Table 1. UH101 was derived from UH100 upon mutagenesis with diethylsulfate. UH302 was derived from strain P400 by selecting, successively, for resistance to phages Tulb (15), Tula (15), and TuII* (22). The resistance markers have not been analyzed genetically; UH302 lacks the major outer membrane proteins OmpC, OmpF, and OmpA. OmpA-specific phages used besides TuII* were K3 (21) and Ox2 (21). The phage λ vector for cloning experiments was λNM816 (47), and the strain used in transfections was 5K.

A crude preparation of colicin L-JF246 (NaCl extract) was prepared from strain JF246 essentially as described by Foulds (18). Because of the temperature inducibility of λNM816 and hybrids derived therefrom, all strains were grown in L-broth (31) at 30°C.

Preparation of phage, bacterial, and plasmid DNA. Phages were obtained by temperature induction of the appropriate lysogens, concentrated with polyethylene glycol 6000 (48), and purified by isopycnic CsCl gradient centrifugation. Phage DNA was extracted with phenol as described by Cole and Guest (14). Bacterial DNA was prepared by the method of Kaiser and Murray (28). The preparation of plasmid DNA was recently described (10). All enzymatic treatments of DNA were as recommended by the manufacturers (Boehringer Mannheim Corp. and New England Biolabs).

DNA labeling and DNA-DNA hybridization methods. Radioactively labeled probe DNA was prepared by treating dephosphorylated restriction fragments bearing the *E. coli ompA* gene with polynucleotide kinase and [γ-^{32}P]ATP (30). DNA fragments in agarose gels were transferred overnight to nitrocellulose filters (44). The filters were baked for 2 h at 80°C in vacuo and then prepared and hybridized by the method of Wahl et al. (46).

Construction of recombinant transducing phages. Appropriate *Eco*RI restriction fragments carrying the *ompA* genes were isolated from preparative agarose gels (0.7%, wt/vol) and purified by DE52 cellulose chromatography (3). Samples (1 μg) were mixed with an equal amount of *Eco*RI-digested λNM816 and ligated with T4 DNA ligase. Transfection of competent 5K was essentially as described by Lederberg and Cohen (29). Plaques formed by recombinant transducing phages were identified by the plaque hybridization technique of Benton and Davis (8), and those showing positive hybridization were purified by stabbing into

TABLE 1. Bacterial strains

Strain	Genotype or description	Origin (reference)
E. coli		
UH99	*thi pyrD gltA galK str trp recA*	*E. coli* W620 (15)
UH100	As UH99, but *ompA*	(10)
UH101	As UH100, but *lac*	This study
UH302[a]	*thi argE proA thr leu mte xyl arg galK lacY str supE*	P400 (42)
JC5484	F'*lac*$^+$/*his trp lac tonA tsx*	P. A. Manning (1)
5K	*hsdR supE*	N. E. Murray (27)
JF246	*Serratia marcescens* colicinogenic for colicin L-JF246	J. Foulds (18, 20)
S. dysenteriae		J. R. Guest
E. aerogenes		A. A. Hancock
S. marcescens		ATCC 13880

[a] This strain is resistant to phages TuII*, TuIb, and TuIa; it lacks the major outer membrane proteins OmpA, OmpC, and OmpF (see text). Nomenclature is according to Bachmann and Low (4).

plates preseeded with 5K and retested. From those plaques, exhibiting a positive hybridization signal after a second round of purification, phage lysates were produced and used to construct lysogens.

Functions of OmpA proteins. Phage inactivation was measured under conditions of excess receptor by using chloroform-killed cells as described previously (15). Since quantitative data were not required the kinetics of inactivation were not measured; 3×10^4 phage PFU per ml were incubated with 5×10^7 cells per ml at 37°C for 30 min. Sensitivity to colicin L-JF246 was tested as described by Foulds (18). Colicin units per milliliter are defined as the reciprocal of the highest dilutions which gave a clear zone of growth inhibition. Strains to be tested as recipients in conjugation were grown at 30°C to stationary phase. The F *lac*$^+$ donor JC5484 was grown at 37°C to about 2×10^8 per ml. The mixture of donor (5×10^7/ml) and recipient (10^8/ml) was incubated for 40 min at 30°C, and appropriate dilutions were plated onto MacConkey lactose agar (Difco Laboratories) containing streptomycin (200 μg/ml).

Envelope preparation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cell envelopes were prepared, subjected to electrophoresis, and stained with Coomassie brilliant blue as described previously (25).

RESULTS

Construction of recombinant λ *ompA* transducing phages. The *ompA* gene of *E. coli* K-12 is located on a 4.3-kilobase *Pst*I restriction fragment carried by the hybrid plasmid pTU100 (10, 24). This fragment was isolated, radioactively labeled, and used to probe for sequence homologies in restriction digests of chromosomal DNA from *Shigella dysenteriae*, *Enterobacter aerogenes*, and *Serratia marcescens*. In all cases a positive hybridization signal was obtained with a single *Eco*RI restriction fragment, and these were approximately 9.5, 7.0, and 8.0 kilobases in size, respectively. (Further details about the molecular characterization of these *ompA* genes will be presented elsewhere, manuscript in preparation.)

Consequently, *Eco*RI fragments of the appropriate sizes were prepared and ligated into the replacement vector, λNM816. The ligation mixture was used to transfect competent 5K and phages derived from recombinant λ *ompA* transducing phages were detected by a plaque hybridization test (8). Plaques giving a positive hybridization signal occurred at a frequency of about 0.2% irrespective of the source of the *ompA* gene. Ultimately, four homogeneous phage lysates were produced for each type of presumptive λ *ompA* transducing phage. Their DNA was extracted and analyzed electrophoretically after digestion with *Eco*RI. All of the isolates contained fragments identical in size to those detected in the preliminary DNA-DNA hybridization study. Furthermore, these fragments all hybridized strongly with the *E. coli* K-12 gene, thereby confirming that the corresponding *ompA* genes had been cloned. The recombinant transducing phages derived from *S. dysenteriae*, *E. aerogenes*, and *S. marcescens* were termed λ *ompA*-Sh, λ *ompA*-En, and λ *ompA*-Se, respectively. All three λ *ompA* phages appear to be completely stable both in the lysogenic state and during propagation. This observation is contrary to the findings of Nakamura and Inouye (34) for the *lpp* gene encoding the lipoprotein of *S. marcescens*. When cloned in a λ vector this gene exhibited great instability and incurred deletions at a high frequency.

Characterization of the OmpA proteins from *S. dysenteriae*, *E. aerogenes*, and *S. marcescens*. The OmpA proteins in envelopes from *S. dysenteriae*, *E. aerogenes* and *S. marcescens* were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1). They were identified by their heat modifiability (Fig. 2), an established property of the *E. coli* K-12 protein, for this shows an apparent molecular weight of 33,000 after boiling in sodium dodecyl sulfate, but appears as a 28,000-molecular-weight spe-

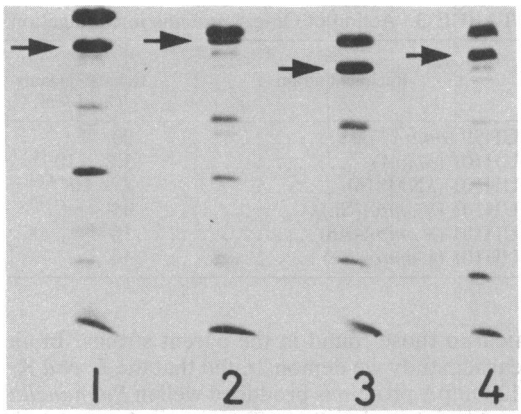

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoretograms of cell envelopes. Lanes: 1, *S. marcescens*; 2, *S. dysenteriae*; 3, *E. coli* K-12 (strain UH99); 4, *E. aerogenes*. The arrows point to the respective OmpA proteins which were identified as described in the text. The prominent band just above each OmpA protein represents the OmpC or OmpF protein (or both) of each strain.

cies when solubilized at temperatures below 60°C (23, 26, 37–39, 41). They also exhibited another unique characteristic (data not shown) of the *E. coli* K-12 protein, namely, partial sensitivity to proteases when these act on cell envelopes. Both trypsin and pronase remove COOH-terminal moieties from the *E. coli* K-12 protein (7, 13, 23, 38, 45), and fragments with apparent molecular weights of 24,000 and 19,000, respectively, remain in the outer membrane. Our findings are essentially in agreement with the results of a comparative study of OmpA proteins performed by Beher et al. (7). However, the apparent molecular weight of the *S. dysenteriae* protein (36,000) is larger than that observed for *Shigella sonnei* and *Shigella boydii* (33,500; 7). Indeed, the mobility of the *S. dysenteriae* OmpA protein is very similar to those of the OmpF and OmpC proteins of *E. coli* K-12 (Fig. 1). One would, therefore, expect to detect the former either in an *ompF ompC* mutant of *E. coli* K-12 or in UH100 (*ompA*) after solubilization of envelope proteins at 50°C.

Expression of the *S. dysenteriae*, *E. aerogenes*, and *S. marcescens ompA* genes in *E. coli* K-12. The partially characterized λ *ompA* transducing phages were used to lysogenize strain UH100. The envelope protein profiles of several strains lysogenic for each of the hybrid phages were analyzed, and representative results are presented in Fig. 2. All three "foreign" OmpA proteins were expressed equally well in *E. coli* K-12 as in their parent strains (Fig. 1). Furthermore, coexpression of the *E. coli* K-12 and *E. aerogenes ompA* genes was demonstrated in the interspecies hybrid UH99 (λ *ompA*-En). Examination of the cell envelope showed both proteins to be present in nearly equal amounts (Fig. 2, lane 7). The problem of the similar mobilities for the *S. dysenteriae* OmpA protein and the *E. coli* OmpF and OmpC proteins was resolved by studying the expression in strain UH302, which lacks the OmpA, OmpC, and OmpF proteins. The heat modifiability of this OmpA protein is also shown.

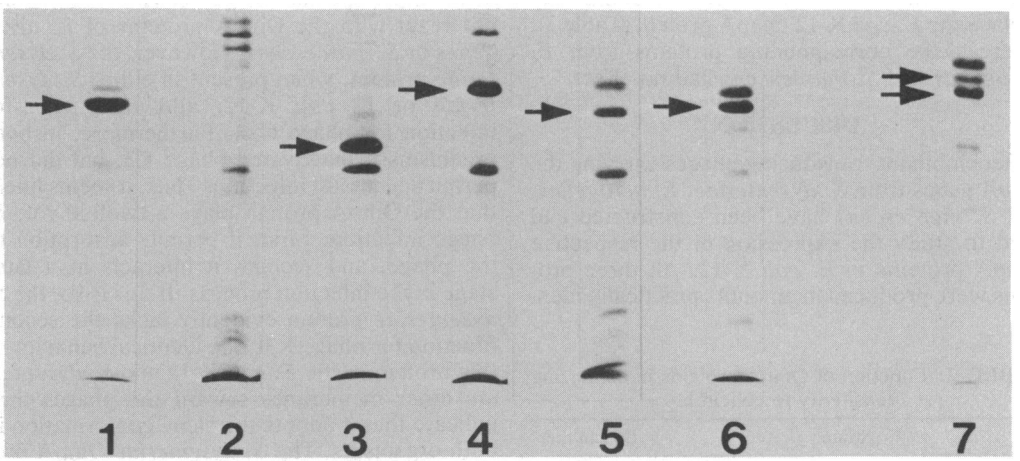

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoretograms of *E. coli* K-12 cell envelopes. Lanes: 1, strain UH100 lysogenic for λ *ompA*-Se; 2, strain UH302 (lacking the OmpC, OmpF, and OmpA proteins); 3, strain UH302 lysogenic for λ *ompA*-Sh and envelopes solubilized at 50°C; 4, the same lysogen as in 3, but envelopes solubilized at 100°C; 5, strain UH99; 6, strain UH100 lysogenic for λ *ompA*-En; 7, strain UH99 (*ompA*$^+$) lysogenic for λ *ompA*-En with both the *E. coli* (lower arrow) and the *E. aerogenes* (upper arrow) OmpA proteins present.

Functions of the OmpA proteins. The isolation of the λ *ompA* transducing phages enabled us to study the properties conferred on *E. coli* K-12 by the foreign OmpA proteins and to compare these with those of the original parent. Lysogens were tested for their sensitivity to the OmpA specific phages Ox2 and K3. All λ *ompA*-Sh lysogens were sensitive to Ox2 but resistant to K3, whereas none of the strains lysogenic for λ *ompA*-En or λ *ompA*-Se showed sensitivity to either of the phages. *S. dysenteriae*, *E. aerogenes*, and *S. marcescens* were tested for their ability to inactivate K3 or Ox2. The latter two did not do so, whereas *S. dysenteriae*, being sensitive to Ox2 and resistant to K3, effectively inactivated both phages at an efficiency of about 20% compared with an *ompA*$^+$ *E. coli* K-12 strain. Furthermore, when the experiment was performed with the interspecies hybrids [*ompA* (λ*ompA*$^+$)] similar results were obtained. In particular, *E. coli* harboring the *S. dysenteriae* OmpA protein could inactivate K3 to the same extent as *S. dysenteriae* itself.

The OmpA protein is required for the action of colicin L. Although it is not the receptor for this molecule (19), it probably allows the colicin to breach the outer membrane. The presence of the OmpA protein from *E. aerogenes* in this membrane rendered *E. coli* K-12 considerably more sensitive to the colicin than its own protein did (Table 2). Both the *S. dysenteriae* and *S. marcescens* proteins were inactive.

F-mediated conjugation in *E. coli* is very inefficient when the recipient cells lack the OmpA protein (40, 42), as OmpA is required for the formation of stable mating aggregates (2). However, the *S. dysenteriae* protein could fully replace the *E. coli* K-12 OmpA protein (Table 3), whereas the corresponding proteins from *E. aerogenes* and *S. marcescens* had no effect.

TABLE 2. Function of OmpA proteins in conferring sensitivity to colicin L

Strain	Colicin titer
UH99 (*ompA*$^+$)	16
UH101 (*ompA*)	<1
UH101 (λNM816)	<1
UH101 (λ *ompA*-Sh)	<1
UH101 (λ *ompA*-En)	128
UH101 (λ *ompA*-Se)	<1

TABLE 3. Action of OmpA proteins in conjugation

Recipient strain	F *lac*$^+$ transconjugants (% of *lac*)
UH99 (*ompA*$^+$)	20
UH101 (*ompA*)	3.6×10^{-4}
UH101 (λNM816)	4×10^{-4}
UH101 (λ *ompA*-Sh)	35
UH101 (λ *ompA*-En)	10^{-3}
UH101 (λ *ompA*-Se)	10^{-3}

DISCUSSION

Recombinant transducing phages carrying the *ompA* genes from *S. dysenteriae*, *E. aerogenes*, and *S. marcescens* have been constructed and used to study the expression of the respective OmpA proteins in *E. coli* K-12. All three proteins were produced in amounts practically identical to those found in the parent strains. In an earlier study we demonstrated that the *E. coli* K-12 OmpA protein is produced well in *Salmonella typhimurium*, but less efficiently in *Proteus vulgaris* (16). It was recently shown that the *ompA* genes from other *E. coli* strains (clinical isolates) are either not or only very poorly expressed in *E. coli* K-12 (6). It therefore seems remarkable that the *S. dysenteriae*, *E. aerogenes*, and *S. marcescens* OmpA proteins are efficiently produced and correctly translocated to the outer membrane of *E. coli* K-12. This suggests that those features of the OmpA protein which are recognized by the protein export system are highly conserved.

The OmpA proteins produced by the genera *Shigella*, *Enterobacter*, *Serratia*, and *Escherichia* cross-react serologically (7). This fact, together with the successful identification of the corresponding genes by DNA-DNA hybridization, is indicative of highly conserved amino acid sequences. This in turn predicts that the various OmpA proteins should share functions; the data presented partially substantiate this. The OmpA specific phages of *E. coli* K-12 did not react with the OmpA proteins of *E. aerogenes* or *S. marcescens*. However, the *S. dysenteriae* protein, when present in either *S. dysenteriae* or *E. coli* K-12, allowed successful infection for phage Ox2. Furthermore, in both organisms it inactivated phage K3, but did not permit successful infection. Thus, it seems likely that the OmpA protein plays a twofold role in phage infection. First, it permits adsorption of the phage, and second, it interacts at a later stage in the infection process. If this is so, the *S. dysenteriae* protein evidently lacks the second function for phage K3. The identical behavior of this protein in the *E. coli* K-12 and *S. dysenteriae* outer membranes toward the phages may indicate that it adopts the same conformation in both organisms. The *S. dysenteriae* OmpA protein could also fully substitute for the *E. coli* K-12 protein in conjugation. Therefore, we assume that this function, which at the biochemical level has remained unknown, is also as highly specific as the phage-phage receptor interaction.

S. dysenteriae, *E. aerogenes*, and *S. marcescens* are resistant to colicin L, but this may be due to the absence of the appropriate receptor. Since nothing is known about the role of the OmpA protein in the action of this colicin, we cannot comment on the rather surprising finding that, in *E. coli* K-12, the *E. aerogenes* OmpA protein renders the cells considerably more sensitive than the *E. coli* protein itself does. However, it is anticipated that this function, too, is highly specific and should be effected by a direct interaction between a domain of the OmpA protein and the colicin-colicin receptor complex.

Experiments are in progress to determine the nucleotide sequences of the cloned genes. It is hoped that these data will not only contribute to an understanding of the evolution of the OmpA protein among the *Enterobacteriaceae* but also provide some insight into its functions, particularly since two of the proteins studied lack some of these functions.

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