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Expression and Regulation of Protein K, an Escherichia coli K1 Porin, in Escherichia coli K-12

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Using a modified lambda phage as a vector and a procedure developed in Dr. C. Schnaitman's laboratory, we have cloned the structural gene for protein K from an Escherichia coli K1 strain to an E coli K-12 strain. The cloned inserts consist of two HindIII fragments, 4 kb and 6.5 kb in size. The protein produced by the insert is nearly identical to "authentic" protein K when chymotryptic peptides of ^{125}I-labeled proteins are compared. Protein K was found to respond to changes in the osmolarity of the medium, being favored in trypticase soy broth (high osmolarity). This fluctuation was not dependent on a functional ompR gene. However, protein K was not expressed in strains carrying the envZ-473 mutation. Thus, protein K appears to be within a class of exported proteins whose expression is regulated by the envZ gene independent of the ompR gene.

Key words: outer membrane protein, porin protein, outer membrane protein expression, outer membrane protein regulation, cloning of porin proteins

The outer membrane of gram-negative bacteria differs from a typical membrane bilayer in that it has an asymmetric distribution of lipids [1]. The inner leaflet of the outer membrane contains phospholipids whereas the outer leaflet has as its lipid constituent lipopolysaccharide. The outer membrane contains transmembrane proteins called porins that serve as pores to allow the diffusion of small, hydrophilic molecules across the outer membrane [2–4]. The porins that are generally found in Escherichia coli K-12 are the OmpF and OmpC proteins. The E coli K-12 cell can also produce other porin proteins. For example, when the cell is starved for phosphate, the PhoE porin protein is induced [5,6]. lc protein is a porin that is expressed when an E coli cell is lysogenized with phage PA-2 [7].

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Protein K is an outer membrane protein that was first described by Paakkanen, Gotschlich, and Makela in encapsulated strains of E coli isolated from patients with neonatal meningitis [8]. This protein is distinct from OmpF protein, OmpC protein, PhoE protein, and Lc protein based on its mobility in sodium dodecylsulfate (SDS)-polyacrylamide gel systems. However, nine out of ten N-terminal amino acids from E coli B protein I and protein K are identical. The result suggests that protein K is structurally related to the OmpF-like porin protein of E coli B.

We extended the structural homology of protein K to porin proteins by comparing chymotryptic peptide maps of protein K to chymotryptic peptide maps of OmpF, OmpC, and PhoE proteins of E coli E-12 [Sutcliffe, Blumenthal, Walter, and Foulds, J Bacteriol 156:xxx, 1983]. Protein K had seven or eight peptides in common with each of these K-12 porins. In addition, the presence of protein K in a porin-deficient strain of E coli K-12 restored the growth rate in minimal medium to that of wild-type E coli. This suggested that protein K functions as a porin in the outer membrane.

In this paper we describe the cloning of the structural gene for protein K from an E coli K1 strain to an E coli K-12 strain. In addition, we show that the expression of protein K in the outer membrane is under the regulation of the envZ gene.

MATERIALS AND METHODS

Bacterial Strains, Phages, and Culture Media

The bacterial strains used in this study are listed in Table I. Most of the strains are derived from E coli K-12. Strain N63-1 is an unencapsulated E coli K1 strain that was isolated as a strain resistant to phage E, a phage that is specific for E coli K1 encapsulated strains [9]. Strain JS17 is an E coli K-12 strain that has been lysogenized with a hybrid lambda vector that carries the structural gene for protein K, a porin protein found in E coli outer membrane. The phage λ540 was given to us by Dr. C. Schnaitman and was constructed by N. Murray as a cloning vector that contains a single HindIII restriction site [10]. Phage K17 was isolated in this study (described below) and is a hybrid λ540 phage that carries the structural gene for protein K.

Phage sensitivity was determined by the cross-streak method or by plating dilutions of phage with 5×10^8 cells/ml. The culture media were L broth (10 gm tryptone, 5 gm yeast extract, 5 gm NaCl, pH 7.0), trypticase soy broth (Baltimore Biological Laboratories), and nutrient broth (Difco Laboratories).

Procedure for Cloning the Structural Gene for Protein K

The procedure for cloning the structural genes of porin proteins was developed by Dr. T. Gregg in Dr. C. Schnaitman's laboratory, University of Virginia, Medical School, Charlottesville, Virginia. The protocol will be described in detail by these workers [T. Gregg, G.A. McDonald, S.S. Cross, W.R. Marcotte, D.J. Conard and C. Schnaitman, personal communication]. Briefly, the procedure can be described in the following manner. Purified DNA from the vector, λ540, and the donor, strain N63-1, are restricted with Hind III and ligated according to manufacturer's instructions (Bethesda Research Laboratories, Bethesda, MD or by [11]). Reconstituted phage, including phages that are potentially carrying the structural gene for protein K, are allowed to infect strain CS146. Because strain CS146 is porin-deficient and unable to allow the diffusion of nutrients across the outer membrane barrier at a normal rate, it grows slowly. Thus, when a phage that has the genetic information to

TABLE I. Bacterial Strains

Strains	Genotype	Source or derivation
CS146	K-12 ompA ompC ompF	C. Schnaitman [12]
N63-1	unencapsulated K1	Derivative of strain N63 [8]
JS17	CS146 protein K^+	This work
MC4100	F^- Δ (lacU-169) aroD-139 rpsL relA tlbB ptsF	T. Silhavy [19]
MH1160	MC4100 ompR-101	T. Silhavy [19]
MH764	MC4100 ompR-4	T. Silhavy [19]
MH1471	MC4100 envZ-473	T. Silhavy [19]
RT3	MC4100 envZ-3	R. Taylor [31]

express a porin lysogenizes cells of strain CS146, the expression of the functional porin will enhance the growth rate of these cells. The result is a larger, more turbid plaque when compared to the plaque resulting from a λ540 phage infection. These larger, more turbid plaques are purified and the phages are used to make lysates on strain CS146. The lysates contain hybrid λ540 phages that carry DNA from the E coli K1 strain N63-1, specifying the synthesis of a porin. Next, this lysate of hybrid λ540 is used to prepare a lysogen. The expression of the porin is confirmed by examining outer membrane protein profiles of the hybrid λ540 lysogens in SDS-polyacrylamide gel electrophoresis and comparing these profiles to the outer membrane protein profiles of strain N63-1 (protein K^+) and the porin-deficient strain CS146. In addition, porin-containing strains are sensitive to colicins E2 and E3 while porin-deficient strains are not sensitive [12].

Peptide Maps

Protein K from strain N63-1 and protein K from strain JS17 were iodinated with $Na^{125}I$ in polyacrylamide gel slices and digested with chymotrypsin [13]. The resulting peptides were separated and compared using two-dimensional (2D) thin-layer chromatography and electrophoresis [14].

Protein K Expression in ompR and envZ Strains

The phage lysate, named K17, carrying the structural gene for protein K was used to lysogenize midexponential cultures of strain MC4100, MH1471, MH1160, MH764, and RT3. Cells from the turbid centers of plaques were picked and streaked for purity. Single-colony isolates were then grown in nutrient broth or trypticase soy broth and the outer membrane protein fractions were prepared as Sarkosyl-insoluble material after breaking the cells by passage through a French press [15].

Gel Electrophoresis

The gel system used to separate protein K from OmpF and OmpC proteins is a 9% SDS-polyacrylamide/6 M urea gel. It is essentially system D [in 16] except 6 M instead of 8 M urea was used.

Electrophoresis of DNA was done in 0.8% agarose gels containing 20 mM TRIS/HCl, pH 8.0, 2.5 mM sodium acetate, and 0.5 mM ethylenediamine tetraacetic acid (EDTA; TAE buffer). The gel also contained 1 μg of ethidium bromide per ml so that the DNA bands could be detected when the gel was exposed to ultraviolet

light. The gels were immersed in TAE buffer and samples were loaded into preformed wells and electrophoresized at 100 V for 3 hr at room temperature.

RESULTS

The source of the structural gene for protein K was purified DNA from unencapsulated E coli strain, strain N63-1. This DNA was completely digested with the restriction endonuclease HindIII. This digest, potentially carrying an intact structural gene for protein K, was ligated into HindIII-cleaved phage λ540 DNA. The ligated phage DNA molecules were packaged into mature phage particles in vitro and this pool of reconstituted phage was used to transduce E coli K-12. When the pool of reconstituted phage was used to lysogenize strain CS146, larger, more turbid plaques were observed at a frequency of one or two per 10^3 plaques. We made the assumption that protein K functions as a porin in the outer membrane and purified putative hybrid lambda phages from the larger, more turbid plaques. We presumed that these phages were carrying the structural genes for porins and prepared lysates of the phages.

These lysates were used to prepare lysogens of strain CS146. Outer membrane fractions were prepared from purified lysogens after growth in 40 ml of L broth. These fractions were electrophoresized on SDS-polyacrylamide gels and the gels were examined for the presence of a predominant protein in the "porin" region (proteins with molecular weights corresponding to 35,000–42,000 daltons). One of the lysogens, designated strain JS17, produced a large amount of a protein in the appropriate molecular weight range. We have designated the hybrid phage which lysogenizes strain JS17 as phage K17.

Data that the protein being produced in strain JS17 functions as a porin has been presented elsewhere [Sutcliffe, Blumenthal, Walter, and Foulds, J Bacteriol 156:xxx, 1983]. In summary, the evidence is as follows: (1) Strain JS17 can grow in minimal medium whereas strain CS146, a porin-deficient strain, cannot. (2) Strain JS17, unlike strain CS146, is sensitive to colicins E2 and E3, indicating the presence of a functional porin in the outer membrane [12].(3) Strain JS17 has an enhanced rate of uptake of nutrients when compared to strain CS146. (4) Purified protein K functions as a porin in black lipid membranes.

We know of no phages or colicins that specifically use protein K as a receptor. Thus, we biochemically compared the porin protein being produced in strain JS17 to "authentic" protein K from E coli K1, strain N63-1. A representation of the composite peptide map from chymotryptic digests of ^{125}I-labeled proteins is shown in Figure 1. The two proteins have identical peptide maps with one exception. Protein K has a peptide with a basic, hydrophilic character that is not seen in the chymotryptic digest of the porin protein from strain JS17. This difference could result from lack of recognition of the appropriate promotor for protein K in E coli K-12, or from a restriction-modification event of K1 DNA by K-12 enzymes or HindIII fragmentation of the N- or C-terminal portion of the structural gene for protein K. Nonetheless, we still conclude that a functional structural gene for protein K has been cloned on this λ540 vector.

The hybrid phage K17 has the ability to integrate at λatt and can be induced with ultraviolet light (UV). A lysate can be obtained after UV induction of strain

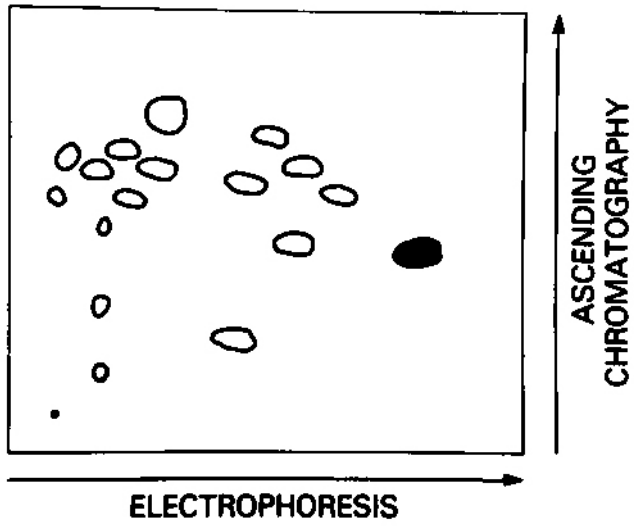

Fig. 1. Chymotryptic peptide map comparison of authentic protein K and porin protein from strain JS17. Protein K from strain N63-1 and porin protein from strain JS17 were purified and radioiodinated as polyacrylamide gel slices following SDS-polyacrylamide gel electrophoresis of the respective outer membrane protein fractions. The radioiodinated proteins were digested with chymotrypsin and the resulting peptides were separated by 2D thin-layer chromatography and electrophoresis. ○, peptides in common; ●, peptide in digest of strain N63-1 protein and not in strain JS17.

JS17. The lysate can be used to prepare other E coli lysogens that produce protein K (see below).

Phage K17 contained a 4-kb and a 6.5-kb insert into the vector HindIII site as shown in Figure 2. We are currently investigating whether both HindIII fragments are necessary for the expression of protein K.

Protein K is a porin protein that functions in a manner similar to the OmpF and OmpC porins of E coli K-12. The expression of the OmpF and OmpC porin proteins of E coli K-12 is in part regulated by the genes of the ompB locus [17–19]. These two genes have been designated ompR and envZ. They function together to alter the relative and absolute levels of the two proteins in response to environmental changes such as the osmolarity of the growth medium [20,21] or the presence of a fermentable carbon source [19]. The porin fluctuation in response to environmental factors can also be elicited by growth in undefined media such as nutrient broth (NB), which favors OmpF expression. In addition, the production of the porins can be affected by alterations of lipopolysaccharide core structure [22] or membrane lipid composition [23,24].

The ompR gene encodes a positive regulatory protein required for the transcription of ompF and ompC. Two classes of ompR mutations have thus far been isolated [19,25]. One confers an $OmpF^{-}OmpC^{-}$ phenotype and the second confers an $OmpF^{+}$ $OmpC^{-}$ phenotype. Mutations in the envZ gene exhibit several phenotypes and show altered regulation of the porins. Some of the envZ alleles, especially those

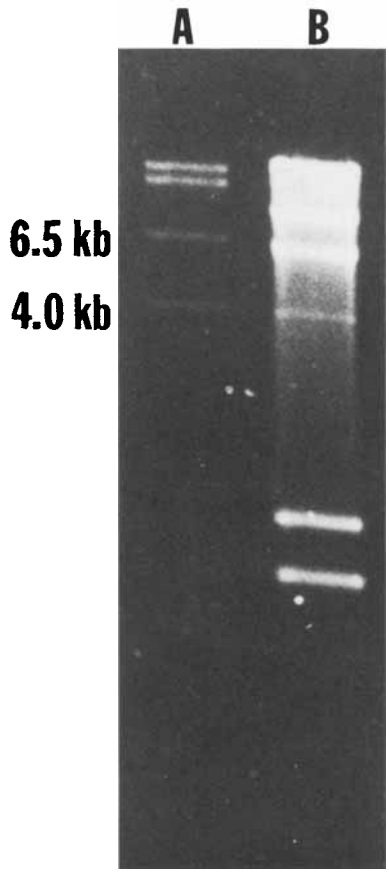

Fig. 2. Gel electrophoresis of K17 DNA. A) DNA from bacteriophage K17 was isolated, digested with HindIII endonuclease and the restriction fragments were separated by electrophoresis in 0.8% agarose gels. The position of the two HindIII inserts of K1 DNA are the 4.0-kb and 6.5-kb fragments. The other two bands represent the two HindIII fragments of vector λ540 DNA. B) HindIII digest of lambda DNA as a reference standard for molecular sizes.

demonstrating an OmpF$^-$ OmpC$^+$ phenotype, are pleiotropic [26,27]. They cause a decrease in the amounts of many other exported proteins that are normally found in the periplasm and outer membrane. Local anaesthetics such as procaine cause a phenotype similar to this class of envZ alleles [28–30]. A second class of envZ alleles prevents the pleiotropic effects caused by local anaesthetics, allowing the expression of exported proteins in the presence of these drugs [31]. This class of envZ alleles is OmpF$^+$ OmpC$^-$ with only a slight effect on other exported proteins during growth in the absence of local anaesthetics.

Since protein K is quite similar to the OmpF and OmpC porins, it follows that the expression of protein K might also be under the regulation of the ompR and envZ gene products. Using phage K17, we made lysogens of the following isogenic E coli K-12 strains: (1) strain MC4100, a laboratory E coli K-12 strain that is ompR$^+$ envZ$^+$ and therefore OmpF$^+$ OmpC$^+$, (2) strain MH1160, an ompR-101 derivative of strain

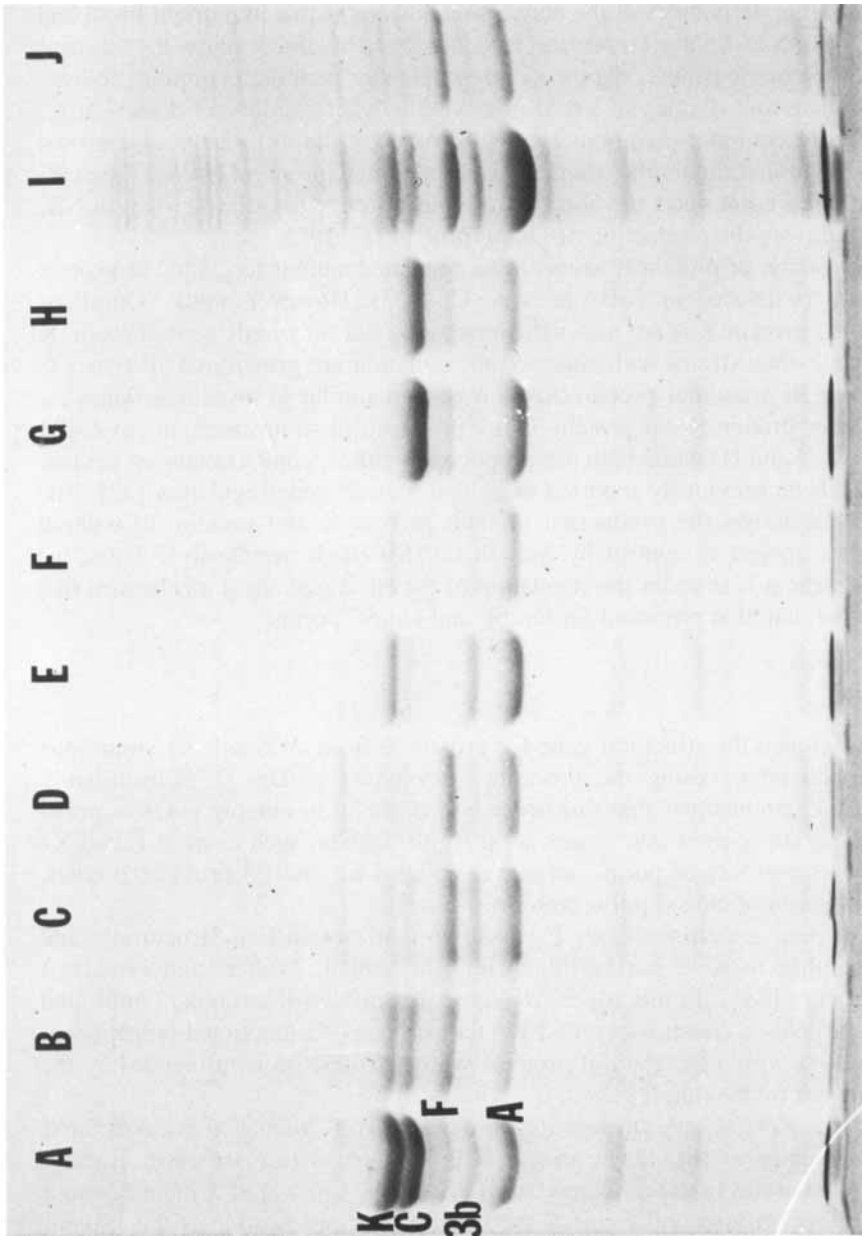

Fig. 3. SDS-polyacrylamide gel electrophoresis of outer membrane fractions from isogenic ompR and envZ strains containing protein K. Lanes A, C, E, G, I: outer membrane protein profiles of strain MC4100, MH764, MH1160, MH1471, and RT3 grown in TSB. Lanes B, D, F, H, J: outer membrane protein profiles of strains MC4100, MH764, MH1160, MH1471, and RT3 grown in NB. The positions of protein K, OmpC protein, OmpF protein, 3b protein, and OmpA protein are designated by K, C, F, 3b, and A, respectively (lane A).

MC4100 that is OmpF$^-$ OmpC$^-$, (3) strain MH764, an ompR-4 derivative of strain MC4100 that is OmpF$^+$ OmpC$^-$, (4) strain MH1471, an envZ-473 derivative of MC4100 that is OmpF$^-$ OmpC$^+$, and (5) strain RT3, an envZ-3 derivative of strain MC4100 that is OmpF$^+$ OmpC$^-$.

The lysogenic derivatives of the above strains were grown in nutrient broth and trypticase soy broth and outer membrane fractions were obtained following Sarkosyl extraction of membrane pellets. Figure 3 represents outer membrane proteins following electrophoresis and staining of a 6 M urea/SDS polyacrylamide gel. Lanes A, C, E, G, and I represent outer membrane protein profiles of the different strains grown in TSB, a medium that favors the production of the OmpC protein [17–19]. Lanes B, D, F, H, and J represent outer membrane protein profiles of the strain grown in NB, a medium that favors the production of OmpF protein [17–19].

The expression of protein K seems to be regulated similar to OmpC as protein K's production is favored in TSB (lanes A, C, E, I). However, unlike OmpF or OmpC proteins, protein K is not under this regulation via the ompR gene. Protein K is still produced when strains with either ompR mutation are grown in TSB (lanes C and E). Protein 3b is another protein that is regulated similar to protein K (lanes A, C, E, I). Neither protein K nor protein 3b are produced in strains with an envZ-473 mutation (lanes G and H) while both are produced in either ompR-containing strains. Protein 3b has been previously reported to be under envZ gene regulation [32]. The envZ-3 mutation allows the production of both protein K and protein 3b without affecting their response to control by growth in TSB (high osmolarity). Thus, we conclude that protein K is under the regulation of the envZ gene by a mechanism that may be different than that proposed for OmpF and OmpC porins.

DISCUSSION

We have cloned the structural gene for protein K from an E coli K1 strain into a hybrid lambda phage using the procedure developed in Dr. C. Schnaitman's laboratory. This demonstrates that this procedure is useful in cloning putative porin genes from different sources into strains sensitive to lambda, such as most E coli K-12 strains. The sensitivity of porin$^+$ strains to colicins E2 and E3 provides a quick check for expression of cloned porin proteins.

From previous experiments, we have shown that protein K is structurally and functionally similar to K-12 porins [Sutcliffe, Blumenthal, Walter, and Foulds, J Bacteriol 156:xxx, 1983]. In this paper, we show that protein K is unlike OmpF and OmpC porin proteins in that it is expressed in the absence of a functional ompR gene. Thus, protein K is within the class of proteins whose expression is influenced by the envZ gene and not by the ompR gene.

Protein K is a major outer membrane protein in E coli strains that are associated with neonatal meningitis [8]. Since protein K is a major surface structure in these strains, it may be useful in the development of a vaccine. Cloning of protein K into a porin-deficient background will facilitate the isolation and purification of this protein for use in production of antibody. Experiments are currently in progress to determine if antibody to protein K promotes phagocytosis in vitro and/or is bactericidal. We are also interested in the potential role protein K may have in K1 capsule biosynthesis.

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