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NOTES

Strong Function-Related Homology between the Pore-Forming Colicins K and 5

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Sequence determination of the *Escherichia coli* colicin K determinant revealed identity with the *E. coli* colicin 5 determinant in the immunity and lysis proteins, strong homologies in the pore-forming region (93.7%) and the Tsx receptor-binding region (77%) of the colicins, and low levels of homology (20.3%) in the N-terminal region of the colicins. This latter region is responsible for the Tol-dependent uptake of colicin K and the Ton-dependent uptake of colicin 5 in the respective colicins. During evolution, the DNA encoding colicin activity and binding to the Tsx receptor was apparently recombined with two different DNA fragments that determined different uptake routes, leading to the differences observed in colicin K and colicin 5 import.

The properties of colicin K (6, 10) and the mode of action of colicin K on cells and in artificial membranes (8, 24) have been studied in detail. Colicin K forms defined channels in membranes that destroy the electrochemical potential of the cytoplasmic membrane, and thus it kills cells. Uptake into sensitive cells requires the outer membrane proteins Tsx, OmpF, and OmpA (20) and the Tol system, composed of TolA, TolQ, TolR, and TolB (25). Colicin K has been purified from spent medium, and the amino acid composition has been determined (6). Although it was confused for some time with colicin A (11), colicin K is one of the well-characterized colicins.

We are interested in colicins because they are suitable tools to study the import of proteins through the outer membrane and the periplasmic space and into or across the cytoplasmic membrane. This process must be efficient since only a few molecules of colicin suffice to kill sensitive cells of *Escherichia coli*. Recently, we characterized colicins 5 (16) and 10 (15), which are unusual since they are taken up via the Ton system, composed of the TonB, ExbB, and ExbD proteins (2, 17), but their cognate Tsx receptor was thought not to belong to the Ton-dependent receptor proteins. Since colicin K binds to Tsx and requires the Tol system for uptake, we compared colicin K with colicins 5 and 10 to see whether their amino acid sequences reveal regions involved in the common Tsx dependence and the distinct Ton and Tol dependence. Furthermore, between colicins K and 5 we observed cross-immunity, which is rather unusual among colicins since even structurally and functionally closely related colicins display distinct immunities (3, 5, 12, 19).

The colicin K determinant was excised from plasmid pColK-K49 (Fig. 1) with *Asp*700I, and the 3.8-kb restriction fragment was cloned into the *Hin*dII site of pBCKS+. *E. coli* 5K transformed with the resulting plasmid pHP50 released colicin K and was immune to crude cell extracts obtained from *E. coli* K49(pColK-K49) and *E. coli* BZB2116(pColK-K235) containing colicin K. Both strands of a 2,161-bp fragment were completely sequenced (Fig. 2), revealing three encoded genes designated *cka* (colicin K activity), *cki* (colicin K immunity), and *ckl* (colicin K lysis). *cka* and *ckl* had the same transcription polarity, and *cki* had the opposite polarity (Fig. 1), an arrangement typical for genes of pore-forming colicins. *cka*, *cki*, and *ckl* encode open reading frames of 548, 96, and 43 amino acid residues, respectively. The colicin produced by transformants expressing the cloned colicin K determinant was active against the same cells as the colicin formed by *E. coli*(pColK-K49): wild-type cells were sensitive, while cells with mutations in *tsx*, *ompF*, *ompA*, or *tolA* were insensitive (Table 1).

Since the literature reports differing molecular weights of colicin K, ranging from 45,000 to 75,000 (9), we performed sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (16) with cell extracts of *E. coli* WM1576(pHP50) (Fig. 3). The molecular weight derived from the electrophoretic mobility was 66,000, which is close to the calculated molecular weight of 59,611. The amino acid composition derived from the nucleotide sequence is very close to the composition determined by amino acid analysis of isolated colicin K-K235 (Table 2).

Comparison of the amino acid sequences of colicins K and 5 (Fig. 4) revealed a low level of homology in the N-terminal region (20.3%), which in all colicins contains the determinant for transport across the outer membrane. The different N-terminal sequences reflect the different uptake routes of colicins K and 5 via the Tol and the Ton systems, respectively, and they reflect the TolC dependence of only colicin 5 (16), as defined previously by comparison of colicins 10 and E1 (15). In contrast, the central region is highly homologous (77% identity), reflecting binding of both colicins to the common Tsx receptor. The level of homology in the pore-forming domains at the C-terminal end is even higher (93.7% identity). Since the C terminus is thought to interact with the immunity protein, cross-immunity between colicins K and 5 is based on the identical sequences in this region, and the distinct sequences of colicins 5 and 10 in this region confer the colicin-specific immunities (16) (underlined in Fig. 4). The 100% identity between the immunity proteins of colicins K and 5 further supports the argument for complete cross-immunity. Although there might be strict constraints on the structure of immunity proteins for specific interaction with their cognate colicins, it is

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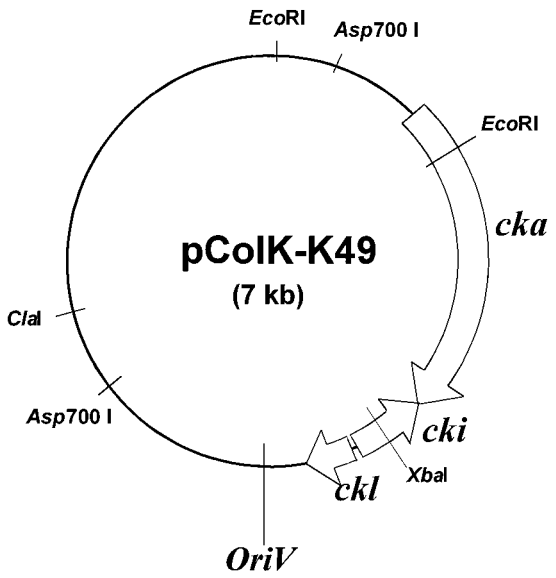

FIG. 1. Arrangement of the colicin K activity (*cka*), immunity (*cki*), and lysis (*ckI*) genes on the natural plasmid pColK-K49. The arrows indicate the transcription polarity. Restriction sites indicated are those used for cloning.

TABLE 1. Sensitivities of *E. coli* strains to colicins K and 5

Strain (genotype)	10-fold dilution resulting in clear (turbid) zone[a]	
	Colicin K	Colicin 5
5K (wild type)	2 (3)	2 (3)
AB2847 (*tsx*)	(0)	(0)
A592 (*tolA*)	r	2 (3)
HP45 (*tonB*)	2 (3)	r
KB426 (*ompA*)	0 (2)	2 (3)
MC513 (*ompF*)	0 (1)	2 (3)
C65 (*tolC*)	2 (3)	r
5K, pHP51 (*cki*$^+$)	(1)	(1)
5K, pHP40 (*cfi*$^+$)	(1)	(1)

[a] The last 10-fold dilutions which resulted in clear zones of growth inhibition and those resulting in turbid zones (in parentheses) are listed. For example, 2 indicates that the colicin suspension could be diluted 10^2-fold to yield a clear zone. r, resistance.

surprising that no conservative amino acid substitutions and even no silent nucleotide replacements occurred in *cki* and *cfi*. The previous failure to recognize cross-immunity between colicins K and 5 (1) was probably due to the loss of pColK-K235 from *E. coli* BZB2116, since also in our hands, the same strain

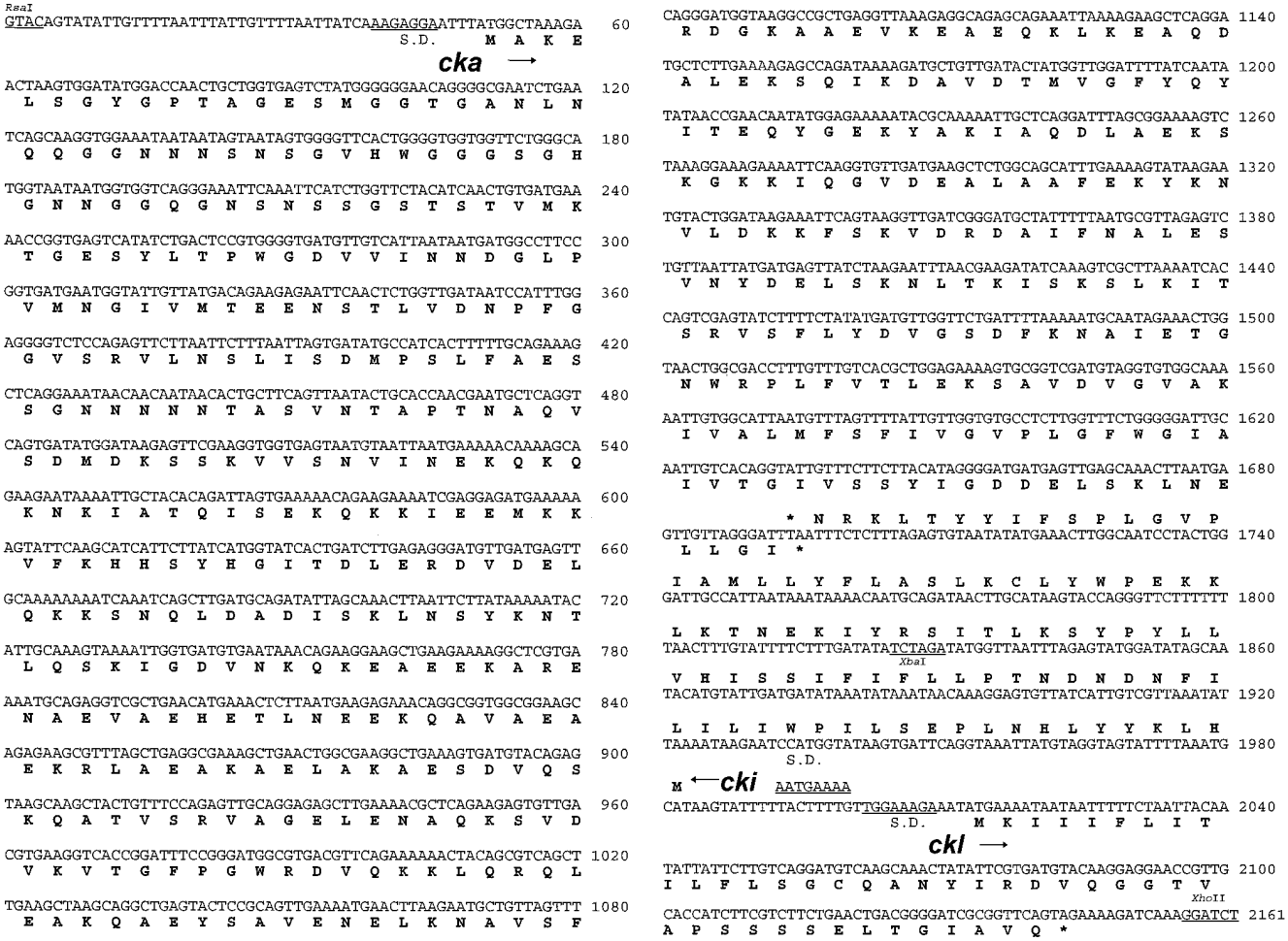

FIG. 2. Nucleotide sequences of *cka*, *cki*, and *ckI*. The predicted Shine-Dalgarno sequence (S.D.) is indicated. This sequence has been deposited in the EMBL nucleotide sequence data library (accession no. X87834).

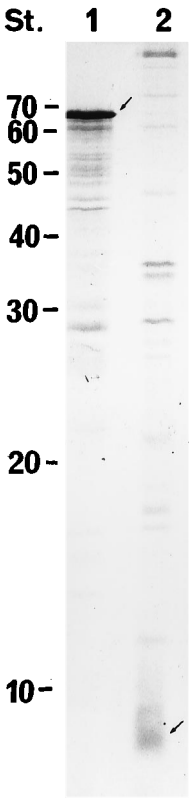

FIG. 3. SDS-PAGE of [^{35}S]methionine-labeled proteins of *E. coli* WM1576(pHP50 *cka*) and WM1576(pHP51 *cki*). The arrows indicate colicin K (lane 1) and the immunity protein (lane 2). The molecular masses (in kilodaltons) of standard proteins (St.) are given.

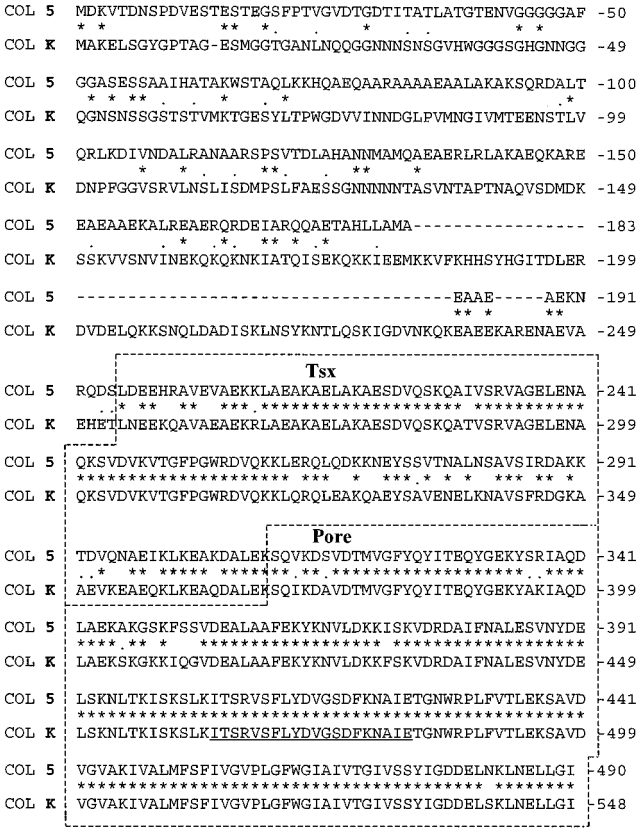

FIG. 4. Comparison of the colicin K and colicin 5 amino acid sequences. Identical residues are marked by asterisks, similar residues are indicated by dots, and dashes indicate sequence gaps that were introduced to optimize the homology. The region proposed to be involved in the Tsx-dependent uptake across the outer membrane and the proposed pore-forming region in the cytoplasmic membrane are boxed.

TABLE 2. Amino acid composition of colicin K[a]

Amino acid(s)	Deduced no. of residues	% of total residues	
		Deduced	Chemically determined
Lys	58	10.58	10.7
His	6	1.09	1.1
Arg	11	2.01	2.2
Asn + Asp	42 + 30	7.66 + 5.47	13.5
Thr	24	4.38	4.6
Ser	50	9.12	9.1
Gln + Glu	27 + 48	4.93 + 8.76	14.5
Pro	9	1.64	1.7
Gly	44	8.03	8.1
Ala	47	8.58	8.7
Cys	0	0	0
Val	46	8.39	7.9
Met	10	1.82	1.5
Ile	25	4.56	4.2
Leu	38	6.93	6.9
Tyr	13	2.37	2.5
Phe	15	2.73	2.8
Trp	5	0.91	0.9

[a] The amino acid content, expressed both in absolute terms and as a percentage of the total number of amino acid residues, was determined from the nucleotide sequence and compared with the chemically determined composition of the purified protein reported by Goebel (6).

from the same source (T. Pugsley, Institut Pasteur, Paris, France) did not confer immunity to colicins 5 and K and also did not produce colicin K. This conclusion is supported by the phenotype of strain BZB2116 pColK-K235, obtained from E. Bremer, University of Marburg, Marburg, Germany, that conferred immunity to colicins 5, K-K235, and K-K49.

The lysis proteins that facilitate release of colicins generally display a high degree of homology but are not identical. However, Ckl and Cfl are identical, and the nucleotide sequences of *ckl* and *cfl* differ only by a single base pair. Since Ckl contains a cysteine residue in the consensus sequence of lipoproteins of the murein-lipoprotein type (7), it is likely that Ckl is modified by the same type of lipid as are the other lysis proteins (22).

Colicins display the same multidomain structure regardless of whether they form channels, act as nucleases, or inhibit lipid carrier regeneration (3, 19). The functional domains are responsible for binding to the receptor (central region), for uptake via the Ton or Tol system (N-terminal region), and for killing cells and binding to the immunity protein (C-terminal region). The uptake region of colicin 10 has been further subdivided into sites involved in Ton- and TolC-dependent transport (15). The data on colicin K support our previous domain assignments in that colicin K shows only a very low level of homology to colicin 5 in the N-terminal region, because it does not require the Ton system and TolC, as does colicin 5. In addition, our proposal that colicin genes are assembled from DNA fragments that encode functional domains

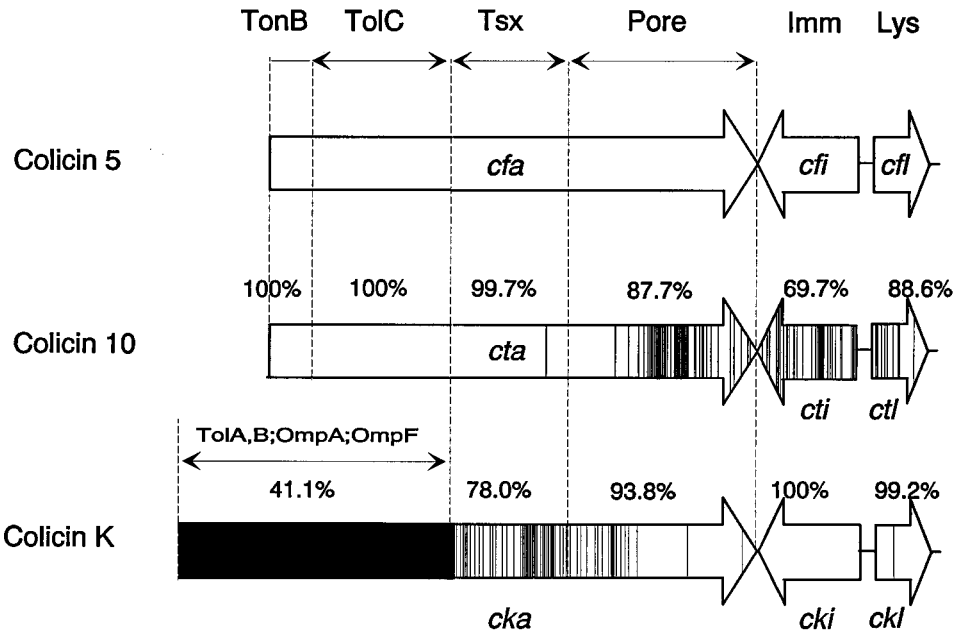

FIG. 5. Differences in the nucleotide sequences, indicated by vertical bars, of the genes encoding the activity, immunity, and lysis genes of colicins K and 10, compared with those of colicin 5. The genes encoding the activity proteins were divided into gene fragments coding for domains binding to the Tsx receptor and pore formation. The 3′ part of *cka* contains so many nucleotide replacements that they were no longer resolved (black box).

(15, 23) is fully supported by the sequences of the colicin K determinant. If one relates the nucleotide sequences of colicins K and 10 with those of colicin 5, the differences are centered in the 3′ half of *cta*, in *cti*, in *ctl*, and in the 5′ half of the *cka* gene (Fig. 5). This pattern of nucleotide substitutions suggests that the DNA fragment encoding the N-terminal half of colicin K is derived from a source different from that of colicins 5 and 10 and that the DNA fragment encoding the C-terminal half of colicin 10 and the immunity and lysis proteins must have an origin other than that of colicins K and 5. The number of amino acid replacements in the N-terminal part of colicin K, which is much higher than that in the central part, suggests different origins of these two DNA fragments. It is remarkable that the various DNA pieces encode rather precise functional domains which when recombined within the homologous regions in different combinations give rise to new colicins.

The plasmids encoding the determinants of colicins K, 5, 10, and E1 are similar in size, and the colicin activity, immunity, and lysis genes are arranged in the same manner as that shown in Fig. 1 for the colicin K determinant. The sequences flanking the colicin determinants are homologous (data not shown), and the common *Eco*RI site of the pCol5, pCol10, and pColK plasmids is located in a gene homologous to the *exc2* gene of pColE1 (4) (data not shown). These features and the amino acid sequences assign colicin K to the group of pore-forming colicins to which colicins E1, Ia, Ib, 5, and 10 belong, as opposed to the class of pore-forming colicins composed of colicins A, B, and N.

We tentatively proposed a TolA box in colicin E1 (15). A similar box is contained in colicin K (underlined in Fig. 6A) located at the beginning of the glycine-rich sequence in the N terminus, which occurs in many colicins (21). Colicin K exhibits an additional high level of sequence homology with a short segment of colicin E3 (13) (Fig. 6A). Furthermore, colicin K displays a strong sequence homology with a small portion of colicin A (14) (Fig. 6B), with which it shares the OmpF and Tol requirement for uptake. After binding to the same Tsx receptor, colicins K, 5, and 10 are translocated across the outer membrane by distinct transport systems. How these transport systems differ mechanistically can only be assessed when the modes of action of the Ton and the Tol systems are understood. The colicins may be suitable tools to unravel these systems.

A

```
          13                                                  53
Colicin K  GESMGGTGANLNQQGGNNNSNSGVHWGGGSGHGNNGGQGNS
           * -  *-* -          * **-********** ** ***
Colicin E3 GGASDGSGWSSENNPWGGGSGSGIHWGGGSGHGNGGGNGNS
          31                                                  71
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B

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          61                                          96
Colicin K  TVMKTGESYLTPWGDVVINNDGLPVMNGIVMTEENS
           **** *-** **** *-**  * * *** *** -**
Colicin A  TVMKPGDSYNTPWGKVIINAAGQPTMNGTVMTADNS
          50                                          85
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FIG. 6. (A) Sequence similarity between colicins K (residues 13 to 53) and E3 (residues 31 to 71) in the regions presumably involved in Tol-dependent uptake across the outer membrane. The previously proposed TolA box (15) is underlined. (B) The sequence similarity between colicins K and A that presumably specifies interaction with the OmpF protein in the outer membrane. Asterisks in both panels denote identical residues; dashes indicate similar residues.

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