INTERNATL MICROBIOL (2000) 3:239–245

RESEARCH ARTICLE

Juan Luis Concepción Curbelo
Maria-Elena Garcia Diaz

Department of Biology, Faculty of Sciences, University of the Andes, La Hechicera, Mérida, Venezuela

The purified colicin S8 is a multimeric protein

Received 15 June 2000
Accepted 15 September 2000

Correspondence to:
Maria-Elena Garcia Diaz. Departamento de Biología. Facultad de Ciencias. Universidad de los Andes. CIGEN. La Hechicera 5101. Mérida. Venezuela
Tel.: +58-74401310
Fax: +58-74401286
E-mail: malena98@telcel.net.ve

Summary Bacteriocins have been isolated both as simple proteins and as proteins in association with carbohydrates, lipids, etc. Colicins are commonly inducible and extracellular. Their molecular masses range from 30 to 90 kDa. Pure colicin S8 was obtained in three steps from supernatant of induced cells: (i) Ammonium sulfate precipitation; (ii) anion exchange chromatography; and (iii) phenyl-Sepharose hydrophobic chromatography, either by preparative or fast performance liquid chromatography (FPLC) analytical purification procedure. In our hands, purified colicin S8 was an aggregation of extremely related polypeptides. Composition of those active fractions was the same: five polypeptides of molecular weight around 55 kDa. Behavior on molecular filtration indicated a molecular weight higher than 200 kDa. Similar results were obtained when purification was carried out through FPLC. Producing strains contain a single plasmid that encodes colicin S8; in minicells, this plasmid was shown to specify a 60 kDa polypeptide. We conclude that more than one form of colicin S8 exists. The forms are structurally related and can be recognized by antibodies raised against one of the polypeptides. Consistent with this conclusion, comparison of peptides produced after hydrolysis with chlorosuccinamide indicated that the active proteins contained both shared and unique components.

Key words *Escherichia coli* · Colicins · Multimeric protein · Polypeptides · Plasmid

Introduction

Colicins are toxic proteins produced by *Escherichia coli* and closely related bacteria, which are active against other susceptible strains in the same group. They are produced in large amounts and, in general, are secreted through the cell envelope to the extracellular medium. They can adsorb to specific receptors located at the external surface of the outer membrane of sensitive cells, and are then translocated to their specific targets within these cells. Colicin S8 was first characterized by Antón [Antón DN (1964) Ph.D. Thesis, Universidad de Buenos Aires, Argentina] and classification as a distinct colicin was based on resistant mutants [9]. Like colicin K, strains made resistant to colicin S8 also show resistance to phage T6. A plasmid, pColS8, determines production of colicin S8. A 2.2 kb region in the plasmid contains the structural gene for the colicin [10]. The mode of action of colicin S8 is similar to some membrane-acting colicins, such as K, A, E1 and Ia [11].

Colicins are commonly inducible and extracellular, both properties being specified by plasmid genes. Their molecular masses range from 30 to 90 kDa. The properties of an isolated bacteriocin may reflect preparative conditions as well as some of its functional properties. Colicin K-K235 is the first to have been purified and characterized [13]. Since then, many bacteriocins have been isolated both as simple proteins and as proteins associated to carbohydrates, lipids, etc. Konisky [18] has stressed that the properties of an isolated bacteriocin may reflect the conditions of isolation.

Structural features of pore-forming colicins E1, Ia, Ib and K are indicative of overall structural assymetry. They contain a high proportion of polar amino acids which suggests that they assume elongated forms in solution to maximize their interaction with aqueous environment. Obviously, these colicins may take on a very different overall structure when integrated into the hydrophobic environment of the cell envelope membranes [19]. Studies on structure–function relationships have shown that colicins have a domain-type structure [7]. The general pattern is that colicin activity resides in the C-terminal portion, while the N-terminal region is involved in translocation across the cell membranes, and the central portion interacts with each cognate outer membrane receptor [19]. The pore-forming colicins show extensive homology in the C-terminal sequence of the polypeptide chain. The most striking feature of these

sequences is the presence of a long uncharged hydrophobic sequence flanked by acidic residues. According to hydrophobicity profiles, this stretch of sequence would be classified as a membrane-spanning sequence [26].

Purification of colicin S8, which remains cell-associated after induction of producing cells, is reported here. At least four different native proteins with colicin activity were found and all of them contained the same polypeptides.

Materials and methods

Bacterial strains and growth conditions *Escherichia coli* K-12 VE831 [25] was used as an indicator strain and for control experiments, and VE832 (VE831 containing pColS8) was used as a source for colicin purification. Bacteria were grown at 37°C in LB medium [25]. Where appropriate, solid media was used for colicin detection. For colicin production, growth was carried out with high-speed rotative shaking during incubation using 2-liter flattened flasks of 25 cm bottom diameter containing 1 liter culture. Induction was at A_{600} of 0.4, with mitomycin C added at 0.4 µg/ml (final concentration).

Titration, assay and characterization of colicin S8 Stab test, quantitative and qualitative titres, and titration with sodium dodecil sulfate (SDS) were carried out as described before [11]. Colicin activity on polyacrylamide gels was detected by laying strips of gel slabs or gel tubes on L-agar plates. These plates were then incubated overnight at 4°C after an indicator strain in soft L-agar was laid on. The plates were incubated at 37°C for 16 h.

Preparation of colicin active fractions Induced cells were harvested by centrifugation (6000 × *g* for 10 min) from 1 liter cultures. After resuspending in 200 ml washing buffer (0.01 M Tris-HCl pH 8.0) treatment was carried out by gently shaking for 3 h at 37°C in the dark. Cells were again harvested, and supernatant saved as WASH. A cocktail of protease inhibitors (10 mM leupeptin, 50 mM phenylmethylsulfonyl fluoride [PMSF] and 100 mM N-a-p-tosyl-L-lysine chloromethyl ketone [TLCK]) obtained from Sigma, was present in buffers.

Polyacrylamide gel electrophoresis and elution of proteins Gradient gel slabs of SDS-acrylamide from 7.0 to 12.5% or 7.0 to 10% were prepared following Goding and Handman [12]. Approximately 60–70 µg protein was mixed with about 20 ml sample buffer and boiled for 5 min. Polyacrylamide gel slabs or tubes were like those described by Davis [6]. After electrophoretic separation of the proteins, gels were either stained with Coomassie blue R-250 or assayed against sensitive bacteria as described above. Glycoprotein staining was carried out according to Fairbankes et al. [8].

Preparative acrylamide gel slabs (2 mm thick), loaded with about 80 µg protein, were horizontally cut using a vertical stained strip of gel to localize protein bands. The desired protein was extracted by electroeluting the 2–3 mm wide gel slice, inside a dialysis bag, on a horizontal electrophoresis apparatus. The extracted protein was removed from the dialysis tubing in a small volume of washing buffer and lyophilyzed in a LABCONCO lyophilyzer.

Ammonium sulfate precipitation Colicin S8 activity was precipitated in 20 to 60% ammonium sulfate from three pooled WASHES at 4°C. The precipitate was centrifuged (12,000 × *g* for 30 min), and a pellet containing colicin activity was dissolved in 20 mM Tris-HCl, pH 8.0 (buffer A) and dialyzed against the same buffer.

Anion-exchange chromatography Diethylaminoethyl (DEAE) -Sephacell (10 × 200 mm) was equilibrated with buffer A and loaded with ammonium-sulfate-precipitated active fraction. After elution, a 0 M–1 M NaCl gradient was applied (continuous or discontinuously) to the column, 30 ml/hr, at 4°C. Active fractions were pooled, extensively dialyzed and concentrated through a PM30 (Amicon) membrane.

Sephadex G-75, G-100, G-150 and G-200 columns Columns (75 × 2.5 cm) were equilibrated and eluted with buffer A, at 15 ml/h, 4°C. The G-200 column was calibrated using gel filtration molecular mass markers: α-amylase (200 kDa), monomeric and dimeric bovine serum albumin (66 kDa and 132 kDa), ovoalbumin (45 kDa) and carbonic anhidrase (29 kDa).

Phenyl-Sepharose chromatography Phenyl-Sepharose CL-4B was packed in a 10 × 40 mm column, equilibrated with buffer A, washed with the same buffer containing 25% ammonium sulfate and eluted with the following eluants: (i) Reverse ammonium sulfate gradient (25–0%) in buffer A; (ii) an ethylene glycol gradient (0–60%) in buffer A.

Fast Performance Liquid Chromatography (FPLC) The following columns purchased from Pharmacia were used: Mono Q HR5/5 (Code No 17-0546-01); Superose 12 (Code No 17-0538-01) and phenyl-SuperoseTM HR5/5 (Code No 17-0519-01). Columns were attached to a Pharmacia gradient FPLC System with a Programmer GP250 plus and two P-500 pumps were used. The effluent was monitored at A_{280} using a UV-M Detector. Flow rate was 0.4 ml/min for all columns. Standard proteins, contained in a Pharmacia molecular mass kit, were used to calibrate the column. These proteins included catalase (232 kDa), aldolase (158 kDa), monomeric and dimeric bovine serum albumin (66 kDa and 132 kDa), β-lactoglobuline (36,8 kDa), and cytochrome *c* (12,4 kDa).

Antiserum preparation, assay and immunoblotting Rabbits were intradermically injected with 350 µg of electroeluted polypeptide 1 (as shown on Fig. 1A, lane 3) with colicin activity from SDS-polyacrylamide gel electrophoresis (SDS–PAGE).

Samples were emulsified with incomplete Freund's adjuvant (0.5 ml) (Sigma). Injection was repeated three times at 7-day intervals. The rabbit was bled on day 30 and the serum obtained was pooled, ammonium sulfate precipitated (50%), dialyzed against 5 mM phosphate buffer (pH 7) and stored at –20°C. Final concentration was 1 mg/ml protein. To test for antibody specificity, colicin S8 (10^{12} particles/ml) was incubated with antibody (final dilution 1/10) overnight at 4°C. Activity tests were carried out with the supernatant of this reaction. Immunoblotting was performed as described in [15]. Colicin samples (30 µg) were electrophoresed on a 7–12.5% gradient SDS–PAGE. Proteins in unstained, unfixed gels were transferred to nitrocellulose and subjected to immunoblotting using a 1:1000 dilution of the antiserum. After incubation with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG), the blot was developed using diaminobenzidine as the chromogen.

Chemical proteolysis of colicin Colicin preparations were subjected to proteolysis with chlorosuccinamide for tryptophan and with cyanogen bromide for methionine. Samples of 10 ml were mixed with sample buffer and loaded on denaturing SDS 7.5–15% gradient polyacrylamide gels. Molecular markers were phosphorylase (91 kDa), bovine serum albumin (66 kDa), ovoalbumin (45 kDa), carbonic anhydrase (29 kDa), soy bean tryptic inhibitor (21 kDa) and lysozyme (14 kDa). Peptidic mapping was carried out according to Cleveland [5]. For data processing we used a computer program algorithm from Dr. Jesus Molinari (Department of Biology, Faculty of Sciences, University of the Andes).

Protein concentrations Protein concentrations were determined by the method of Lowry et al. [23], with bovine serum albumin as standard.

Sugar determination Sugar determination was based on the phenol-sulfuric acid method as described by Ashwell [2], using 0.5 mg of protein. A Hewlett-Packard 8452 diode array UV-Vis spectrophotometer was used.

Results

Preparation of a cell-free extract containing colicin S8 activity It was found that optimum induction of colicin S8 was obtained after 60 s exposure to UV-irradiation or with the addition of mitomycin C followed by 3 h incubation at 37°C in the dark. The culture supernatant contained 42% of the colicin activity. Cells continued to produce colicin when they were pelleted, resuspended in isotonic buffer and then incubated in the dark. After three hour-long washes, production of colicin was still significant. A concentrated crude preparation of colicin S8 was obtained by ammonium sulfate precipitation of three pooled washes. This fraction contained almost 100% of the total activity and was named "concentrated crude extract" (CCE).

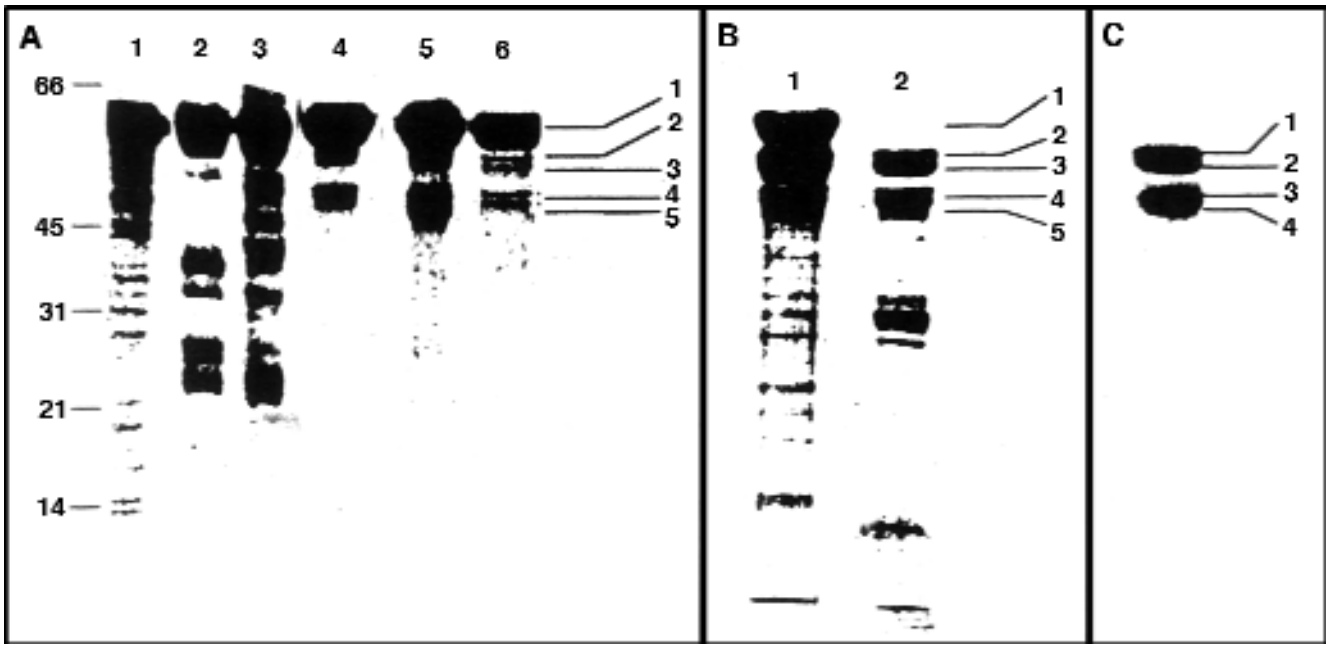

Fig. 1 Polypeptide composition of colicin S8 active fractions. Coomassie blue stained SDS–PAGE gels showing analysis of colicin S8 active fractions. (A) Lanes: 1, WASH; 2, DEAE 0.1 M NaCl fraction; 3, DEAE 0.2 M NaCl fraction; 4, top PAGE gel band; 5, medium PAGE gel band; and 6, bottom PAGE gel band. (B) Lanes: 1, active fraction eluted on a G-150 Sephadex; 2, active fraction eluted on a G-200 Sephadex. (C) Active fraction from phenyl-Sepharose. Numbers at the left represent the molecular sizes (in kDa) of the standard proteins. Numbers at the right represent the molecular sizes (in kDa) of the colicin polypeptides. Microsoft Corel Draw 7 Computer Software was used to compose gel panels

Chromatographic separation by DEAE-Sephacel chromatography Colicin S8 activity in the CCE was divided into two major activity peaks after anionic interchange chromatography: 0.1 M NaCl and 0.2 M NaCl (unshown results). Electrophoretic analysis by SDS–PAGE indicated that some polypeptides are common in both fractions (Fig. 1A, lanes 1, 2 and 3). Thus, two forms of the colicin molecule differing in electronegativity were separated.

We examined 0.2 M active fraction on PAGE, where at least three active proteins could be detected by Coomassie blue staining and activity tests. These proteins differ in both size and electrical charge, as migration on PAGE is a function of these variables. All three active proteins were electroeluted from that gel and then run on SDS–PAGE. Figure 1A, lanes 4, 5, and 6, show five polypeptides that could be identified as: 47, 49, 52, 56 and 63 kDa. These results suggest that active colicin fraction comprises various polypeptides which form macromolecules with distinct electrical charges.

Chromatography on phenyl-Sepharose Total active 0.2 M NaCl-fraction, obtained from DEAE–Sephacell was applied to a phenyl-Sepharose column. An ammonium sulfate reverse gradient (25–0 %) eluted one peak containing colicin S8 activity (unshown results). Electrophoretic analysis of that peak shows it is composed of the polypeptides 2 (56 kDa), 3 (52 kDa), 4 (49 kDa) and 5 (47 kDa) identified above (Fig. 1C). Consequently, colicin S8 contains hydrophobic domains, which allow its retention by phenyl groups. Hydrophobicity of this colicin is consistent with its action on the cell membrane. Furthermore, we can conclude that band 1 is not necessary for activity. This band may represent a less hydrophobic polypeptide which is eluted by reverse gradient ammonium sulfate, compared with the other four polypeptides which show stronger interaction with phenyl groups.

Behavior of the active fractions in molecular filtration (Sephadex) When CCE is chromatographed on columns of Sephadex G-75, G-100, G-150 or G-200, activity elutes in the void volume. This step with G-200 results in considerable loss of activity. SDS–PAGE analysis of the active fractions following Sephadex filtration gives a similar pattern of five polypeptides for each, except for the G-200 fraction. This results are partially shown in Fig. 1B, for G-150 eluted fraction (lane 1) and for G-200 (lane 2). Polypeptide 1 has disappeared after molecular filtration on G-200 (lane 2 in Fig. 1B). This fact, together with the data from chromatography on phenyl-Sepharose and SDS–PAGE analysis, suggests that the active molecule consists of polypeptides 2, 3, 4 and 5, held by hydrophobic forces. A conformational difference would explain the distinct behavior of these polypeptides in phenyl-Sepharose (with activity) and G-200 (without activity).

FPLC analytical purification An attempt to purify colicin S8 using an FPLC system gave the same results. Bearing in mind the above results, it was decided to follow a four step strategy: ammonium sulfate precipitation, anion exchange chromatography, molecular filtration and phenyl-Sepharose hydrophobic chromatography. CCE preparation of colicin produced two major active peaks (0.0–0.1 M NaCl) after a cationic inter-

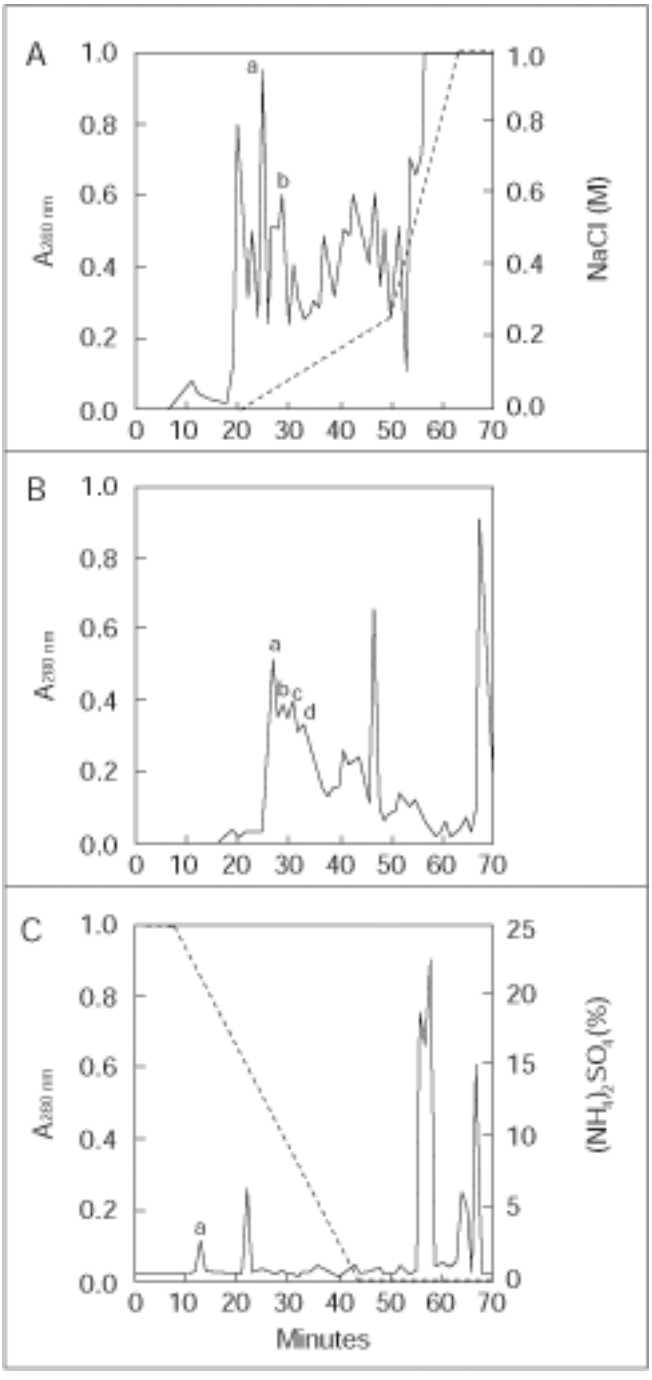

Fig. 2 Analytical FPLC purification. (A) Cationic interchange (Mono Q) chromatography. (B) Molecular filtration (Superose) of the pooled two peaks shown in A. (C) Reverse-phase chromatography (phenyl-Superose) of the pooled four peaks shown in B. Letters a, b, c and d indicate colicin activity detected

Table 1 FPLC analytical purification of the colicin S8

Fraction	Total volume (ml)	Total protein (mg)	Protein (mg/ml)	Total colicin units[a] (U)	Specific activity (U/mg)	Fold purification	Yield (%)
WASH	25.0	1425.0	57.0	2.50×10^8	17.5×10^4	–	100.0
Ammonium sulfate	16.0	800.0	50.0	1.60×10^8	20.0×10^4	1.14	64.0
MonoQ	11.2	16.80	1.5	0.56×10^8	333.3×10^4	19.04	22.4
Superose	2.0	1.20	0.6	0.20×10^8	1250.0×10^4	71.42	8.0
Phenyl-Superose	1.5	0.45	0.3	0.15×10^8	3333.0×10^4	190.47	6.0

[a]Quantitative titres as described before (see Materials and methods).

change column: Mono Q (Figure 2A). These two peaks pooled gave four peaks with activity by molecular filtration: Superose (Fig. 2B). After a reverse phase column: phenyl-Superose, one peak was eluted with ammonium sulfate (Fig. 2C). Data from molecular standards indicate that the protein has a molecular mass of 110–120 kDa. This matrix is intended for use in hydrophobic interaction chromatography. Thus, the hydrophobic character of colicin S8 is demonstrated by its affinity for the resin in the absence of salt.

The SDS–PAGE electrophoretic pattern can be seen in Fig. 3A. Several polypeptides, three of them highly stained, with molecular weights ranging from 47kDa to 63 kDa, are separated on the gel. They correspond to polypeptides 1, 2, 3, 4 and 5, described previously.

FPLC purification results are summarized in Table 1. A purification factor of 190 times was reached and no activity loss occurred after G-200 equivalent molecular filtration (Superose 12).

Immunological comparison of polypeptides from colicin S8 active protein The resulting gel (Fig. 3A) was blotted on nitrocellulose and probed using rabbit antisera to polypeptide 1 (as identified in Fig. 1A, lanes 1 to 6; see Materials and methods). Figure 3B indicates that our polyclonal antibody strongly recognized polypeptides different from polypeptide 1, which are likely to constitute the active protein colicin S8. Phenyl-superose active fraction continues to react with the antibody in spite of the absence of polypeptide 1. Thus, the active form of colicin S8 does not require polypeptide 1. Besides, colicin S8 activity is completely neutralized by the polyclonal antibody in in vitro assays.

Chlorosuccinamide hydrolysis To assess primary structural changes of colicin molecules, polypeptides 1, 2 and 3 were cut off the SDS–PAGE, and proteolytic digestion was carried out with chloro-succinamide (Fig. 4). Although the digestion pattern remains quite similar for all three, there is a larger peptide in the first protein (polypeptide 1) and two smaller peptides in

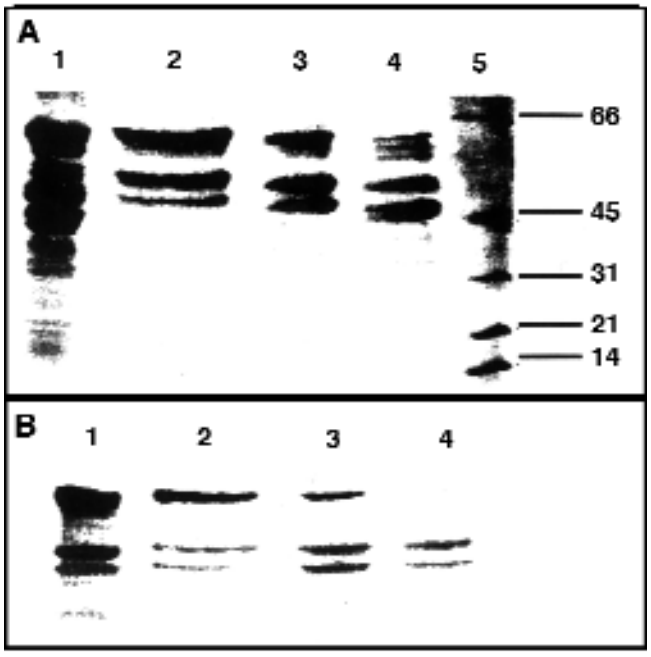

Fig. 3 Analysis of colicin activity from FPLC. (A) SDS-PAGE of active fractions from FPLC. Lanes: 1, WASH; 2, Mono Q; 3, Superose; 4, phenyl-Superose; 5, molecular standard proteins (in kDa). (B) Immunoblotting of gel shown on panel A. Lanes 1, 2, 3 and 4 are identical as in panel A

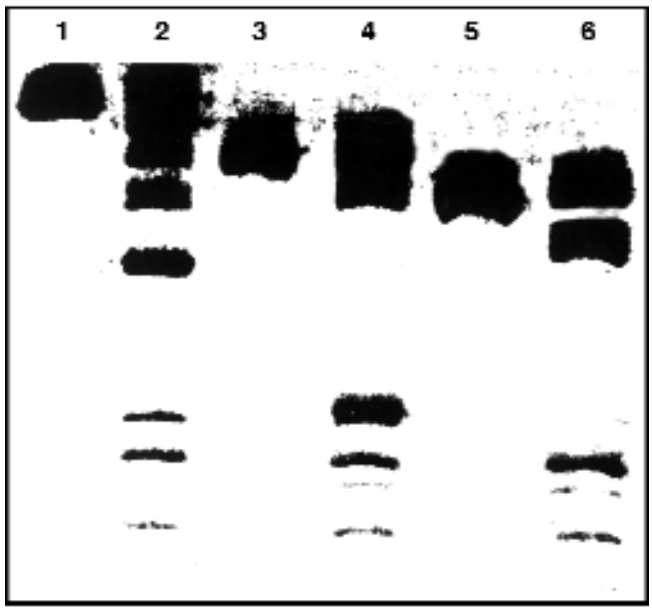

Fig. 4 Cleavage of three different colicin S8 polypeptides by chlorosuccinamide. Coomassie blue-stained SDS-PAGE gel showing analysis of colicin S8 polypeptides. Lanes: 1, undigested polypeptide 1; 2, digested polypeptide 1; 3, undigested polypeptide 2; 4, digested polypeptide 2; 5, undigested polypeptide 3; 6, digested polypeptide 3

Table 2 Physicochemical properties of purified colicin S8

Native molecular size (kDa)	110–120
Subunits	5
Molecular size of subunits (kDa)	47, 49, 52, 56 and 63
Average isoelectrical point	7,4
Heat treatment (% active after 8 min at 80°C)[a]	50%
Detergent treatment (% active after 0.1% SDS overnight at 4°C)	100%
Reducing treatment (% active after 1 mM 1,4 dithiothreitol (DTT), 5% mercaptoethanol overnight at 4°C)	100%
Maximum absorbance	280 nm
Sugar composition	None

[a]Colicin S8 (20 μg/ml) is 50% inactivated after 8 min at 80°C in phosphate buffer.

the third protein (polypeptide 3). Similar results were obtained when cyanogen bromide was used for proteolysis. Analysis of this data [5] gives very low values for $P(x)$ (< 0.01) indicating that all three polypeptides are closely related. This suggests an alteration which may expose new peptidic sites susceptible to cleavage and result in the disappearance of others.

Characterization of the protein colicin S8 Properties of pure colicin S8 are shown in Table 2. Absorbance spectra is as expected for a non-chromogenic protein. No traces of either hexoses or glycosil groups were detected by either of the two methods used: phenol-sulfuric acid reaction [2] and sugar specific gel staining [8].

Discussion

In our experiments, purified colicin S8 was an aggregate of closely related polypeptides. Thus, colicin S8 was purified in three steps from WASH: (i) Ammonium sulfate precipitation; (ii) Anion exchange chromatography; and (iii) phenyl-Sepharose hydrophobic chromatography, either by preparative or FPLC analytical purification procedure.

Colicin S8 is released by the induced cell like other colicins. A general permeability change should occur and a lysis protein (*lys* gen) is necessary [14]. Phospholipase A of the outer membrane plays a role in lysis protein functioning [28]. The lysis gene has been localized on the plasmid [10]. It has been suggested that lysis proteins are assembled in high-molecular weight polymers or micelles, soluble in the culture medium, contributing to colicin release [3]. With colicin S8, once the cells are induced, colicin production continues for 3 h, at nearly the same rate. Titres of colicin activity do not decrease after 3 washes of 1 hour each. Thus, changing the conformation of the cytoplasmic colicin may be either a necessary step or a consequence of the release of the colicin. Thus, we may have purified released and intracellular colicin.

Functional mapping of pColS8 has shown a 1.7 kb region necessary for colicin production, predicting a molecular mass of approximately 57kDa, or maybe 55 kDa, as the lysis protein of about 20 aminoacids is also encoded in that region [10]. In fact, we have identified a polypeptide of 56 kDa as a component of colicin S8, along with polypeptides of molecular mass ranging from 47 kDa to 63 kDa. These smaller polypeptides are not detected in minicells of the producing strain, where only one polypeptide can be seen [10]. Moreover, our results with molecular filtration (Sephadex) suggest a molecular mass above 200 kDa and a 110 kDa protein is purified from FPLC phenyl-Sepharose column.

An overestimated molecular size can be a consequence of having a glycosilated protein [1]. This might be the case if the active conformation of colicin S8 is a dimeric glycoprotein of 110 kDa, the size of the molecule in FPLC. Reports of bacteriocins isolated from genera outside the Enterobacteriaceae usually suggest that there is a single glycoprotein bacteriocin; for example: pertucins [16], morganicin [30] and lactocin [31]. Bacteriocins isolated from genera within the Enterobacteriaceae are usually not glycoproteins, although some colicins have been found to be associated with the glycoprotein O-antigen [18]. In fact, glycosil groups were not detected (unshown results). We suggest that colicin S8 is synthesized as a polypeptide of about 56 kDa. A population of these polypeptides is slightly modified and they form aggregates or a multimeric molecule according to environmental conditions. We do not know if the proposed changes occur in the cells or if they are a consequence of the purification procedure. Our preparation suffers considerable loss of activity after elution from Sephadex columns, which could be explained by a relationship between activity and degree of aggregation: three polypeptides in that case. A difference in conformation between extracellular and cytoplasmic (dimeric) colicin A was found by Knibiehler and Lazdunski [17]. They hypothesized that a hydrophobic polypeptide region which confers competence for membrane binding and to form dimers on the cytoplasmic colicin A becomes masked in the interior of the extracellular protein [22]. The active 110 kDa molecule obtained in FPLC may correspond to the dimeric conformation favored by hydrophobic interactions.

On the other hand, colicin S8 seems to be a member of the ion channel-forming family of colicins [11], proteins that undergo a transition from a soluble to a transmembrane configuration. The channel-forming ability of these proteins resides in their C-terminal portion, which is highly hydrophobic

and inserts in the membrane [29]. Since the molecules are highly soluble in water, this domain should fold as an "inside-out" membrane protein in water [27]. A model has been proposed for the formation of the channel by colicins [21]. Conformational changes are involved in the interaction with the membrane and molecularity of the channel is likely to be one or two molecules/channel [29].

Our proposal is that the 56 kDa polypeptide is the colicin S8 protein and is active on the membrane in a dimeric form (110 kDa). Polypeptides other than the 57 kDa are generated after colicin biosynthesis and they form heteromultimers. G-200 Sephadex might exclude preferentially the most active conformation (and/or combination).

There are reports of multiple forms of colicin K [13] and colicin V [20]. More recently, colicins A, E1 and Ia [24] have been found in vitro in at least two different membrane-associated states. Thus, existence of colicins in more than one conformation seems to be a general phenomenon and reports of a single species may be the consequence of purification of a selected form of the bacteriocin.

The differences found in a chain polypeptide size: five polypeptides from 47 to 63 kDa could be the result of proteolysis. Proteolytic mapping supports this idea. Moreover, all of them are recognized by the policlonal antibody arised from polypeptide 1, only. Recently, it has been demonstrated that colicin A cleavage occurs during both entry into and release from cells by the OmpT protease [4]. These changes could be non-specific.

The question of whether the possible conformational change of colicin S8 has any biological origin and/or physiological significance is adressed in a different paper.

Acknowledgments This research was supported by the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT) Grant S1-1866. We thank Dr. Diane Bohannon for her comments and suggestions during the preparation of this manuscript.

References

1. Andrews P (1965) The gel-filtration behaviour of proteins related to molecular weights over a wide range. Biochem J 96:595–606
2. Ashwell H (1976) In: Morel CM (ed) Genes and antigens of parasites. A laboratory manual. 2nd ed. UNDP-World Bank-WHO eds. Fundação Oswaldo Cruz, Rio de Janeiro
3. Cavard D (1992) Colicin A and colicin E1 lysis proteins differ in their dependance on sec A and sec Y gene products. FEBS Lett 298:84–88
4. Cavard D, Lazdunski C (1990) Colicin cleavage by OmpT protease during both entry into and release from *Escherichia coli* cells. J Bacteriol 172:648–652
5. Cleveland DJ, Fischer SG, Kirshn MW, Laemmli UK (1977) Peptide mapping by limited proteolysis in sodium dodecyl-sulphate and analysis by gel electrophoresis. J Biol Chem 252:1102–1106
6. Davis BJ (1964) Disc electrophoresis. II. Method and application to human serum problems. Ann NY Acad Sci 121:404–427
7. DeGraaf FK (1979) Mode of action of colicin E2, colicin E3 and Cloacin DF13 Zbl Bakteriol Parasitenkd Infektionskr Hyg Abt I Orig Reibe A 244:121–134
8. Fairbankes G, Steck TL, Wallach DFH (1972) Electrophoretic analysis of major polypeptides of human erythrocite-membrane. Biochemistry 10:2606–2671
9. Fredericq P (1946) Sur la classification des *B. coli* d'aprés leurs caractères antibiotiques. Compt Rend Soc Biol 140:1055–1057
10. Garcia M-E, Dyke K (1993) Physical and functional characterization of the ColS8 plasmid. Gene 124:87–92
11. Garcia M-E, Nágel de Zwáig R, Puig J (1982) Mode of action of colicin S8. J Gen Microbiol 128:973–979
12. Goding, Handman (1983) Electrophoretic analysis of protein antigens. In: Morel CM (ed) Genes and antigens of parasites. A laboratory manual. 2nd ed. UNDP-World Bank-WHO eds, Fundação Oswaldo Cruz, Rio de Janeiro
13. Goebel WF, Barry GT (1958) Colicin K. II. The preparation and properties of a substance having colicin K activity. J Exp Med 107:185–209
14. Hakkart MJ, Veltkamp E, Nijkamp HGG (1981) Protein H encoded by plasmid CloDF13 involved in lysis of the bacterial host. 1. Localisation of the gene and identification and subcellular localisation of the gene H product. Mol Gen Genet 183:318–325
15. Harlow E, Lane D (1988) Immunizations. In: Antibodies: A laboratory manual. Cold Spring Harbor Laboratory Press, New York, pp 53–135
16. Kawai Y (1974) Purification and characterization of pertucin produced by *Pseudomonas pertucinogena*. Antimicrob Agents Chemother 6:347–359
17. Knibiehler M, Lazdunski C (1987) Conformation of colicin A: apparent difference between cytoplasmic and extracellular polypeptide chain. FEBS Lett 216:183–189
18. Konisky J (1978) The Bacteriocins. In: Ornston L, Sokatsh JR (eds) The Bacteria, vol. 6. Academic Press, London, pp 71–136
19. Konisky J (1982) Colicins and other bacteriocins with established modes of action. Annu Rev Microbiol 36:125–144
20. Kucharzewska T, Stepien H, Gasior E (1975) Isolation and some properties of colicin V preparations. Acta Microbiol Polonica 8:169–187
21. Lakey JH, Massotte D, Heitz F, Faucon J-F, Dasseux J-L, Parker MW, Pattus F (1991) Conformation of the pore-forming domain of colicin A in its membrane-bound form. Eur J Biochem 196:599–607
22. Lazdunski CJ, Baty D, Geli V, Cavard D, Morlon J, Lloubes R, Howard SP, Knibiehler M, Chartier M, Varenne S, Frenette M, Dasseux J-L, Pattus F (1988) The membrane channel-forming colicin A: synthesis, secretion, structure, action and immunity. Biochim Biophys Acta 947:445–464
23. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
24. Mel SF, Falick AM, Burlingame AL, Stroud RM (1993) Mapping a membrane-associated conformation of colicin Ia. Biochemistry 32:9473–9479
25. Nágel de Zwáig R, Vitelli-Flores J (1973) Mode of action of a new colicin. FEBS Lett 29:318–322
26. Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, New York
27. Pattus F, Massotte D, Wilmsen HU, Lakey J, Tsernoglou D, Tucker A, Parker MW (1990) Colicins: Prokaryotic killer-pores. Experientia 46:180–192
28. Pugsley AP, Schwartz M (1984) Colicin E2 release: lysis, leakage or secretion? Possible role of a phospholipase. EMBO J 3:2393–2397
29. Slatin SL, Raymond L, Finkelstein A (1986) Gating of a voltage-dependent channel (colicin E1) in planar lipid bilayers: The role of protein translocation. J Membr Biol 92:247–254
30. Smit JA, DeKlerk HC, Coetzee JN (1968) Properties of a *Proteus morganii* bacteriocin. J Gen Microbiol 54:67–75
31. Upreti GC, Hinsdill RD (1973) Isolation and characterization of a bacteriocin from homofermentative *Lactobacillus*. Antimicrob Agents Chemother 4:487–494