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Change of Thermal Stability of Colicin E7 Triggered by Acidic pH Suggests the Existence of Unfolded Intermediate During the Membrane-Translocation Phase

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ABSTRACT **Purified colicin E7 was analyzed by CD spectrum and gel filtration chromatography in a mimicking membrane-translocation phase. It was found that the CD spectra of colicin E7 at pH 7 and pH 2.5 were similar. Although the melting temperature of the protein shifted from 54.5°C to 34°C at low pH, the thermal denaturation curves of colicin E7 at different pH conditions still fit a two-state model. These experimental results imply that a minor structural change, triggered by acidic pH, for instance, may reduce the energy required for protein melting. In contrast to the minor change in secondary structure at different pH conditions, we observed that, in vitro, all monomeric colicin E7s converted into multimer-like conformations after recovering from the partial unfolding process. This multimeric form of colicin can only be dissociated by formamide and guanidine hydrochloride, indicating that this protein complex is indeed formed by aggregation of the monomeric colicins. Most interestingly, the aggregated colicins still perform in vivo bacteriocidal activity. We suggest that in a partial unfolding state the colicin is prepared for binding to the specific targets for translocation through the membrane. However, in the absence of specific targets in vitro these unfold intermediates may therefore aggregate into the multimeric form of colicins. Proteins 32:17–25, 1998.** © 1998 Wiley-Liss, Inc.

Key words: colicin E7; CD spectrum; chromatography; protein folding

INTRODUCTION

Colicins are plasmid-encoded, antibiotic-like bacteriocins, produced by *Escherichia coli* that kill some closely related bacteria.[1–5] The E group colicins [6,7] binding to the vitamin B_{12} receptor[8] have well-documented bactericidal activities: colicin E1 forms an ion channel on the membrane,[9] colicin E3, E5, and E6 exert RNase activity,[10] while colicin E2, E7, E8, and E9 exhibit DNase activity[11–13] to sensitive cells. Interaction of various colicins and their cognate immunity proteins have been seen as a powerful model system for the investigation of protein–protein interactions.[14] Recently, interactions between the surfaces of colicin and its cognate immunity protein have been suggested.[15] Moreover, studies of the unusual mechanisms for the secretion of colicin from host cells and for translocation into sensitive cells have drawn a great deal of attention, both in academic and industrial research.[16]

Colicins kill susceptible cells in three steps, and the three distinct domains of colicin coincident with the functions of each step have been defined.[17] First, the receptor binding domain located at the central region of colicin binds to the specific receptor (BtuB for the E group colicins) on the outer membrane; a conserved pentapeptide sequence, EEKRL, can be found in the receptor binding domains of colicin E1 (but not colicin A) and all enzymatic colicins (from colicin E2 to E9). Second, translocation across the membrane is directed by the N-terminal translocation domain with the aid of the Tol system, including OmpF, TolA, TolB, TolQ, and TolR proteins located in the membrane of a susceptible cell.[16,18] An octapeptide "TolA box" is commonly located at the N-terminus of both enzymatic and pore-forming colicins.[19] Finally, the C-terminal domain responsible for the lethal activity refolds and reaches the specific targets of the susceptible cell.

An unfolded state is known to be required for translocation of the protein into or out of the bacteria membranes.[20] The "insertion-competent" conformation has been proposed as a prerequisite for membrane insertion of the pore-forming colicins.[21–23] Conformational change of pore-forming colicins during the membrane-translocation transition has been described in detail.[21–23] Using the channel-forming domain of

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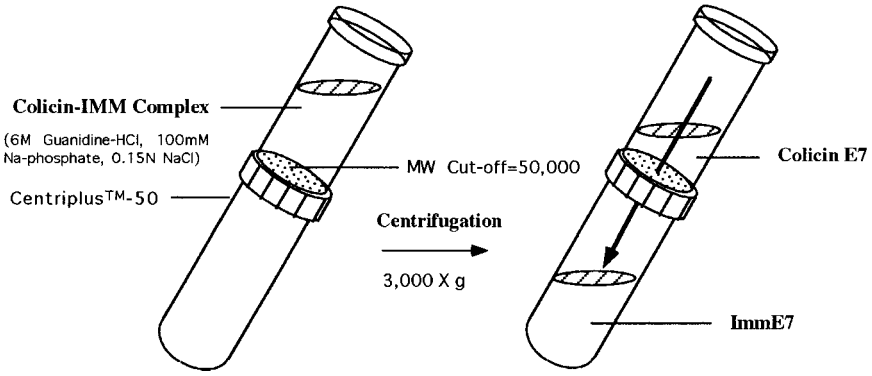

Fig. 1. Separation of the Colicin-Imm complex: The denatured colicin E7-Imm complex is separated by centrifugation with Centriplus(tm) 50 tube. After centrifugation, colicin E7 protein retents in the upper part of the tube and immunity protein is collected in the lower part of the tube.

colicin A and colicin E1 for investigation in vitro, two theories, "molten globule"[21] and "compact unfolded,"[23] have been hypothesized for the mechanism of translocation. A similar conclusion has also been proposed by in vivo assay with intact polypeptide of colicin A.[22] It seems clear that both intact colicin and the truncated channel-forming domain would process a partial unfolded conformation before the aqueous-to-membrane phase. Based on the crystal structure of colicin Ia,[24] a tertiary structural flip-flop or rearrangement model has recently been proposed. According to this newly proposed model, destroying and rebuilding the global structure seems to be omitted.

In this report, prediction of the secondary structure of colicin E7 was obtained from circular dichroism spectroscopy. Our experimental results suggest that a minor alteration of a structure of the protein in membrane translocation phase may lead to the formation of an aggregation-sensitive folding intermediate. Consequently, this intermediate may overcome the energy barrier required for translocation.

MATERIALS AND METHODS

Preparation of Colicin E7 and Its Cognate Immunity Protein

E. coli W3110 (ColE7-K317) has been described previously.[13] Cells were grown at 37°C in L broth and the culture was induced with mitomycin C (0.5 μg/ml) when the OD_{600} of 0.5 was reached. About 4 h after induction, supernatant of the culture was collected. The supernatant was slowly titrated with 3 M sodium acetate (pH 5.0) to a final concentration of 0.1 M, followed by adding ammonium sulfate to the final concentration of 30% (w/v). The mixture was then incubated at 4°C for at least 2 h before removing the insoluble impurities by centrifugation at 20,000*g* for 30 min at 4°C. Then the colicin E7 complex was precipitated at 50% saturation of ammonium sulfate. The colicin complex was pelleted and then resuspended in 100 mM sodium phosphate buffer (pH 7.0) containing 6 M guanidine hydrochloride. The mixture was then incubated at 4°C overnight. Colicin E7 and its immunity protein were separated by centrifugation with Centriplus(TM) 50 tube (Amicon Inc.). Colicin E7 (61.349 kDa) was retained in the upper sample reservoir while ImmE7 (9.926 kDa) passed through the molecular sieve into the filtrate vial (see Fig. 1). Both colicin and immunity proteins were desalted by Sephacryl 200 gel filtration column (2 × 70 cm, Pharmacia). Protein purity was assayed on 15% SDS-PAGE.

CD Spectroscopy

All CD measurements were made on a Jasco J-720 spectropolarimeter interfaced to a PC microcomputer. Temperature was controlled by a JTC-340 temperature control program and Neslab RTE-111 temperature controller linked by an RS232 interface. Temperature scans were performed continuously from 15°C to 90°C in a 1 mm cylindrical water-jacketed quartz cell (Hellma Scientific).

In Vivo Bacteriocidal Activity Assay and Protease Digestion of Colicin E7

20 μl of equal amounts of purified monomeric and aggregated colicin was spotted onto a 0.8 cm blank disk, which was placed on an LA plate overlaid with 2 ml of 0.65% soft agar seeded with 20 μl of sensitive *E. coli* cells. The plates were then incubated at 37°C for 6–12 h. The clear zones appeared around each disk indicate the bacteriocidal activity (DNase-type bacteriocin) of the tested colicins.

Purified monomeric and multimeric fractions of colicin (0.3 mg) in 10 mM Na-phosphate buffer (pH 7.0) were adjusted to pH 2.5 with 1 M Gly-HCl (pH 2.5) at 37°C for 10 min. The protein was then readjusted to pH 8.0 with 2 M Tri-HCl (pH 8.0) followed by trypsin (15 μl of 20 μg/ml stock solution) digestion in a total volume of 130 μl for 10 and 20 min at 37°C. The reaction was terminated by boiling with SDS-PAGE sample buffer. The trypsin digestion patterns of colicins were resolved by SDS-PAGE.

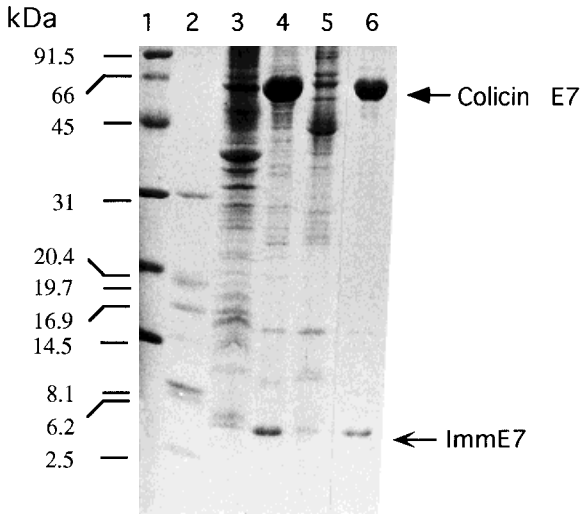

Fig. 2. Purification of colicin E7 complex: Protein contents were resolved in 15% SDS-PAGE and visualized by staining with coomassie blue. The standard proteins are shown in lane 1 and 2, with their molecular masses indicated in kilodalton. Lane 3, proteins remaining in the cell pellet after centrifugation. Lane 4, proteins secreting into the medium were collected by precipitating with 10% of trichloroacetic acid (w/v). Lane 5, shows the miscellaneous proteins accompaning with colicin were removed at the first run of acidic salt-out (0.1 M sodium acetate, pH 5.0; 30% of ammonium sulfate). Lane 6, the purity of colicin after the second run of salt-out (50% of ammonium sulfate) was checked. 100 µl of cultures of *E. coli* strain W3110 (pColE7-K317) growing in L broth containing 0.5 µg/ml mitomycin C at 37°C were used for each lane. The positions of colicin and immunity proteins on the gel are also shown.

RESULTS

Purification of Colicin E7 and Its Immunity Protein

Most colicins produced by cells are released into medium through a semi-selective system.[25] Four hours after induction, only about 10% of colicin E7 was retained in the intracellular fraction (Fig. 2, lane 3). In contrast, a larger amount of colicin E7 and its cognate immunity protein were accumulated in the culture media (Fig. 2, lane 4). The miscellaneous proteins secreted along with colicin were removed by the first run of salt-out (30% ammonium sulfate saturation) at the acidic condition (Fig. 2, lane 5). The colicin complex was precipitated by another run of salt-out at 50% ammonium sulfate saturation (Fig. 2, lane 6). The predicted Mr value of ImmE7 is 9.926 kDa.[13] The size of a monomeric ImmE7 was further confirmed by chromatography (Fig. 5B). However, the size of this protein looks much smaller visualized on SDS-PAGE (Fig. 2). Recently, the crystal structure of monomeric ImmE7 has been reported.[15] It was found that ImmE7 is a 4-helix bundle and highly negatively charged. Perhaps this abnormal mobility of ImmE7 on SDS-PAGE may be due to the unusual structure of the protein.

To dissociate the colicin complex, 100 mg of purified colicin E7 complex was dissolved in 10 ml of sodium phosphate buffer (100 mM, pH 7.0) containing 6 M guanidine-HCl and 0.15 N NaCl. Dissociation of colicin and its cognate immunity was performed as described in Materials and Methods. However, the purified colicin E7 fraction generally gave two peaks at 280 nm (Fig. 3) when guanidine-HCl was removed by column chromatography. The estimated Mr values of these two peaks analyzed by FPLC were 65,000 and approximately equal 200,000, respectively (Fig. 8B). Both peaks exhibited in vivo bacteriocidal activity (Fig. 4) and gave a single band corresponding to 62 kDa on SDS-PAGE (Fig. 3). Thus, the results indicated that these two peaks correspond to monomeric and multimeric colicin E7, respectively. Apparently, multimeric colicin E7 exerted weaker bacteriocidal activity than that of the monomeric protein (Fig. 4). The homogeneity of the proteins were checked and found that both monomeric and multimeric colicin E7 were very stable under these experimental conditions. Perhaps the multimeric colicins need to be dissociated into its monomeric form during the translocation process. This putative dissociation process may therefore weaken the bacteriocidal activity of the protein. Possibly the weak bacteriocidal activity may simply be due to the less efficient diffusion of the multimeric colicins from disk to the surrounding environment.

A single peak derived from the ImmE7 fraction was located between the peaks of chymotrypsinogen (25 kDa) and ribonuclease A (13.7 kDa) markers analyzed by FPCL (Fig. 5A). The estimated Mr value of the ImmE7 was about 20 kDa (Fig. 5A), indicating that the ImmE7 protein is in a dimeric form. In contrast, in the presence of 0.5 M guanidine hydrochloride the ImmE7 was eluted after the ribonuclease marker (Fig. 5B), showing that most dimeric ImmE7s convert into monomeric form (9.926 kDa) under this condition. These results thus imply that a large proportion of ImmE7 exhibits dimeric form in normal physiological condition.

Solvent-Enhanced Dissociation of Multimeric Colicin E7

Some solvents, such as dimethyl sulfoxide (Me2SO), isopropanol, and formamide, were used to investigate the forces involved in the association of multimeric colicin E7. We found that colicin E7 could not be solubilized in 40% (v/v) of Me2SO or isopropanol. Moreover, most monomeric colicin E7 converted to multimeric form when the protein was precipitated with Me2SO or isopropanol (Fig. 6A). Formamide was the only solvent tested that was able to dissociate the multimer to monomer of colicin E7. As shown in Figure 6B, multimeric colicin E7 was quantitatively converted to its monomeric form by incubating the protein in 50% (v/v) formamide at 50°C for 60 min.

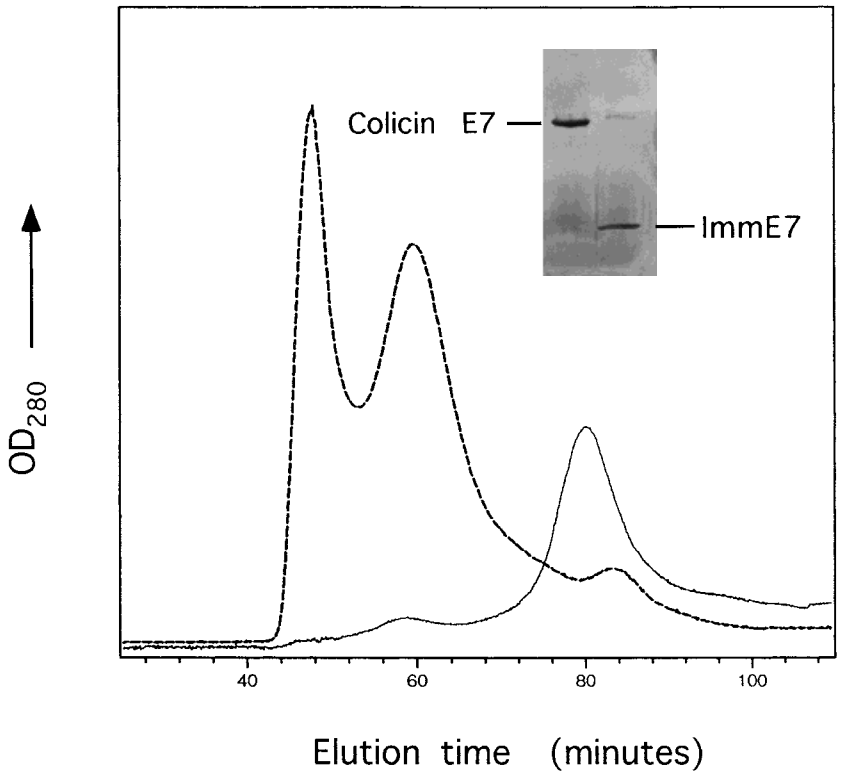

Fig. 3. Separation profiles of colicin E7-Imm complex by Sephacryl G200 chromatography: The Colicin E7-Imm complex was denatured with 6 M guanidine-HCl. The proteins were desalted by Sephacryl G200 chromatography. The purified colicin E7 shows two peaks (dashed line), and the purified ImmE7 gives a single peak (solid line) in the elution profile of chromatography at 280 nm. Purity of these two fractions were checked by silver-stained SDS-PAGE (inserted figure).

Property Change of Colicin E7 Induced by Acidic pH and Temperature

The pH-dependent effects on triggering the translocation competent state and changing the hydrodynamic properties of pore-forming colicins have been described previously.[26–29] The CD measurement of colicin E7 at the far-UV spectra is shown in Figure 7. Deduced from the ellipticity at 222 nm, it was found that the major signature for "-helical conformation of colicin E7 only dropped from 28% to 25% (Table I), while the pH value shifted from 7.0 to 2.5. Apparently these two CD spectra are similar (Fig. 7A), indicating that a large amount of the secondary structure of this protein is maintained even at lower pH. When colicin E7 was denatured by heating to 90°C for 15 min, we found that colicin E7 unfolded to a unique reference state both at pH 7.0 and pH 2.5 (Fig. 7A). These results imply that colicin E7 has a native-like folding and adopts a similar unfolding pathway at low pH conditions.

The thermal unfolding curves of colicin E7 were further examined in solution at acidic and neutral conditions. The profiles of the thermal unfolding curves of colicin E7 are shown in Figure 7B. We observed that these two curves are fitted into a

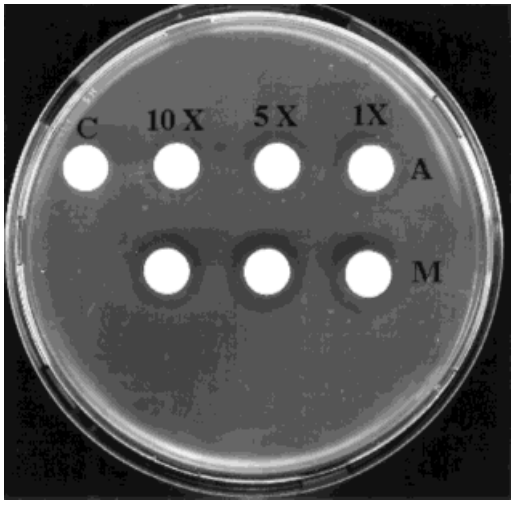

Fig. 4. In vivo bacteriocidal activity assay of the purified colicin E7: Monomeric and multimeric colicin E7 were purified as described in Materials and Methods. Proteins were adjusted to the same concentration. Each protein was then made 1x, 5x, and 10x fold dilutions, and 20 µl of each dilution was used for in vivo bacteriocidal activity test (see Materials and Methods). A: monomeric colicin E7; M: multimeric colicin E7; C: dish without colicin as control.

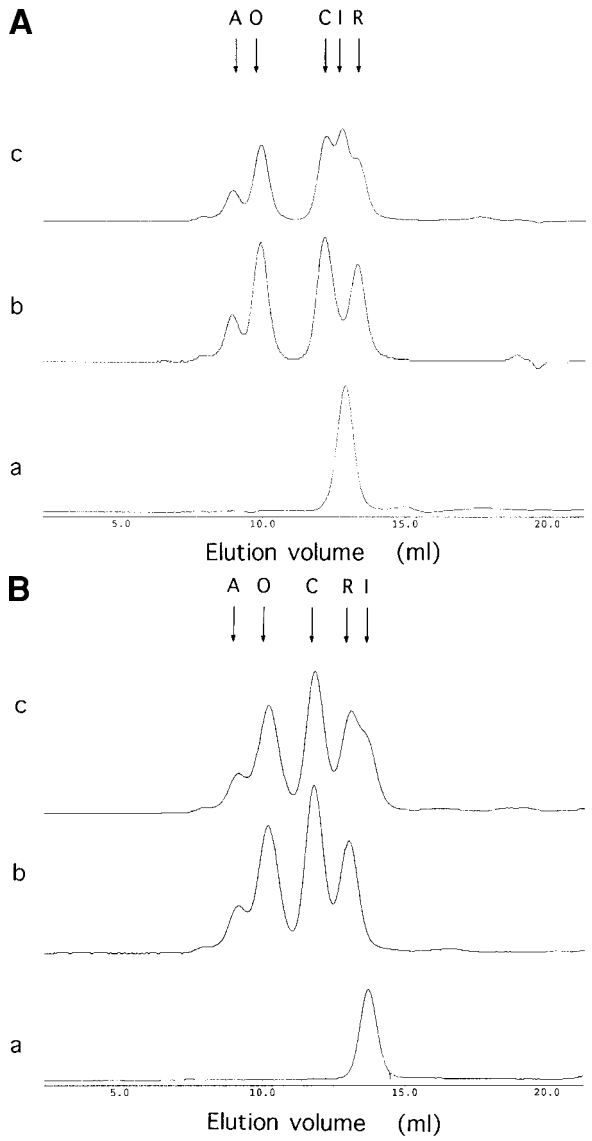

Fig. 5. Size exclusion chromatography of purified ImmE7: The ImmE7 protein obtained (see Fig. 1) were applied to a gel-filtration Superdex 75 HR 10/30 column equipped with Unicorn FPLC system (Pharmacia Biotech Co.). (**A**) At V = 0, 200 µl samples were injected into column equilibrated in sodium phosphate buffer (50 mM; pH 7.0, containing 0.15 N NaCl) at 1.0 ml/min speed; elution of protein was monitored at 280 nm. (**B**) Both column and samples were equilibrated in the buffer containing 0.5 M guanidine hydrochloride. Panel a, ImmE7 only; panel b, protein markers; panel c, elution profile of protein mixture containing ImmE7 and four commercial protein markers. Positions of markers and immunity protein are indicated: I: ImmE7 (1 mg/ml); A: bovine serum albumin (66 kDa, 0.2 mg/ml); O: ovalbumin (43 kDa, 0.45 mg/ml); C: chymotrypsinogen (31 kDa, 0.28 mg/ml); and R: ribonuclease A (13 kDa, 0.426 mg/ml).

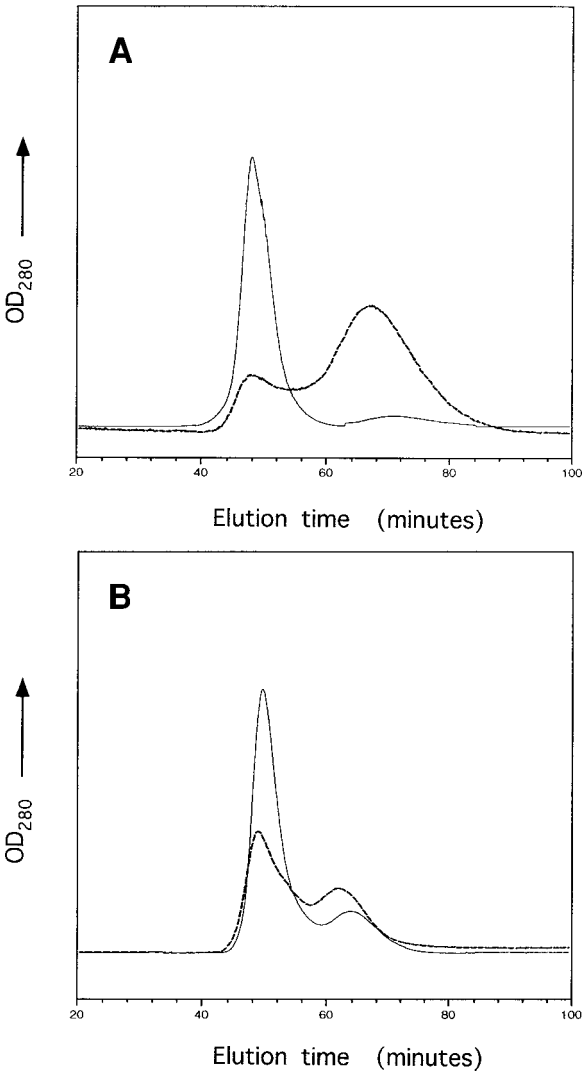

Fig. 6. Gel-filtration chromatography elution profiles of colicin E7 obtained from different solvent conditions: (**A**) Colicin E7 (62 kDa) fractions mainly containing the monomeric proteins from Figure 3 were treated with (solid line) and without (dashed line) isopropanol and loaded into a Sephacryl G200 column. For this treatment, 1 ml of colicin E7 solution (1 mg/ml) containing 100 mM sodium phosphate (pH 7.0), and 0.15 N NaCl was gently mixed with 1 ml of 2-isopropanol. Colicin E7 was thoroughly precipitated by incubation at 4°C for 10 min, then the pellet was resuspended in 1 ml of buffer. (**B**) Colicin E7 fractions mainly containing multimeric proteins from Figure 3 were treated with (dashed line) and without (solid line) formamide and loaded into the Sephacryl G200 column. For this treatment, 1 mg of colicin E7 was suspended in 1 ml of phosphate buffer with or without formamide (50 % w/v) and incubated at 50°C for 1 h.

"two-state transition" model.[30] Surprisingly, the melting temperature (Tm) estimated from the thermal denaturation curves changed from 54 to 34.5°C. Most likely, the intense decrease in Tm makes it easier for colicin E7 to enter into the unfolding state, commonly known as the translocation competent stage.

The deduced secondary structure composition of colicin E7 from the CD spectra was calculated by the method of Yang.[31] The predicted secondary structure revealed that colicin E7 exhibited a slight secondary structural change at low pH conditions (Table I),

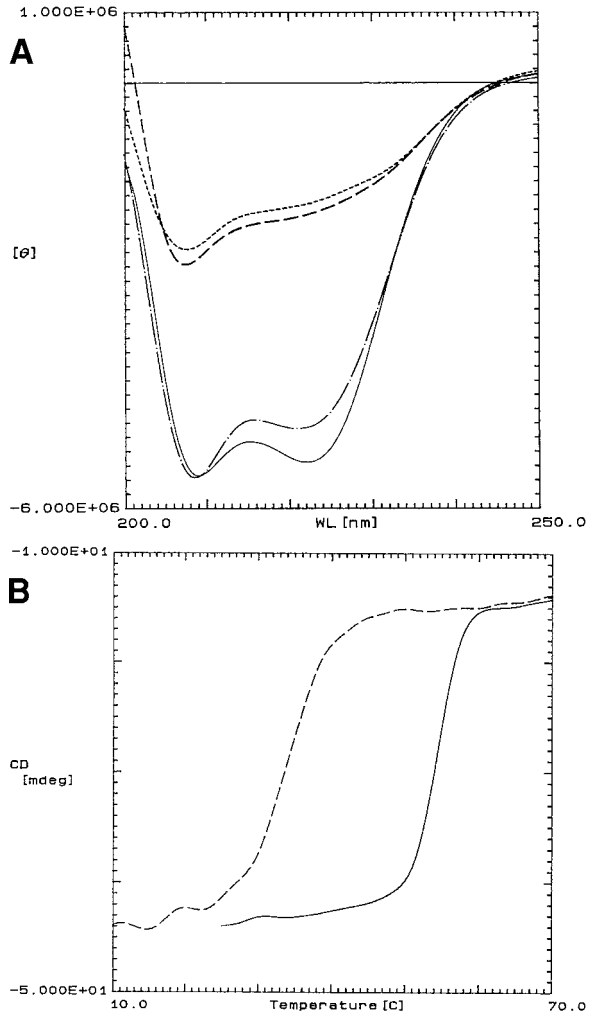

Fig. 7. Acid-denaturation CD spectra of colicin E7: (**A**) The molar ellipticity [2] of CD spectra for colicin E7: at 25°C, pH 7.0 (solid curve) and pH 2.5 (center dotted curve); at 90°C, pH 7.0 (dashed curve) and pH 2.5 (dotted curve). Changes in secondary structure were monitored by scanning from 200 to 250 nm. (**B**) Temperature-dependent CD spectra (222 nm) for colicin at pH 7.0 (solid curve) and pH 2.5 (dashed curve). Monomeric colicin E7 was dissolved in 50 mM sodium phosphate and the pH adjusted with formic acid. All spectra represent the average of three scans using protein concentrations of 0.25–0.5 mg/ml. Bandwidth, 1 nm; full-scale sensitivity, 10 medg; time constant, 2s; and cell path-length of 1 mm. 2, ellipticity (mdeg); [2], molar ellipticity (deg*cm^2*decimole^{-1}).

while the thermal stability of the protein seemed to drop sharply (Fig. 7B).

The Partial Unfolded Intermediate of Colicin E7 May Aggregate Into Multimeric Form in Aqueous Phase

Oligomerization of colicin A[27] and increase of the hydrophobicity of colicin E3[29] can be induced at low pH states. The substantial decrease of thermal stability of colicin E7 at low pH environment has been demonstrated (Fig. 7B). To investigate whether a

TABLE I. Secondary Structure Prediction of Colicin E7*

Conditions	α-Helix	β-Sheet	Turn	Random
	%			
pH 2.5	25.1	13.3	23.3	38.3
pH 7.0	28.0	13.2	20.6	38.2

*The percentage of secondary structure was calculated from the CD spectra by Yang's method.[31]

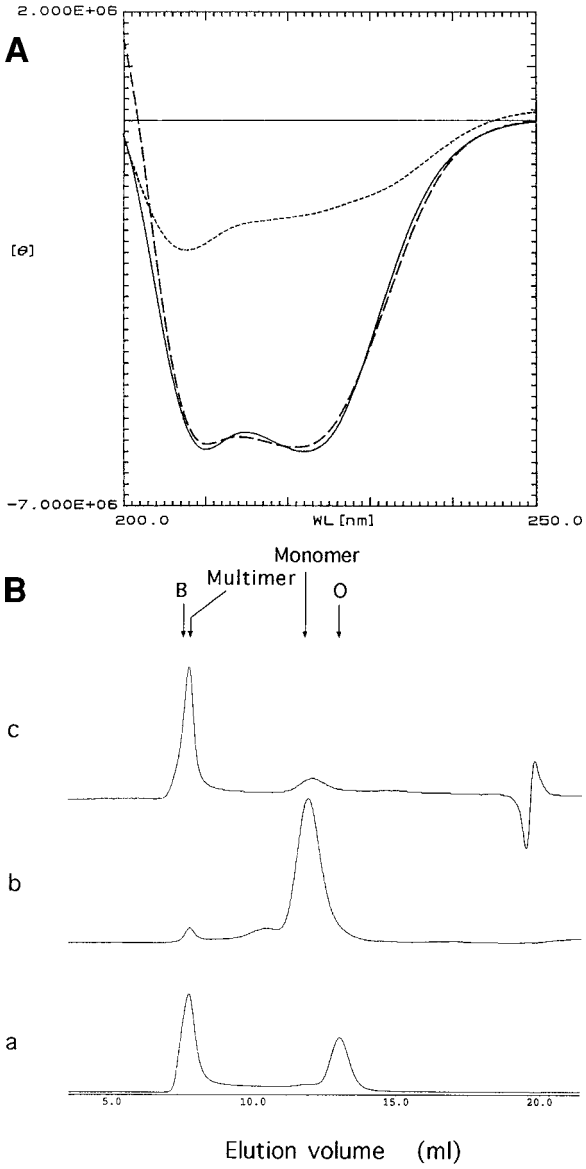

Fig. 8. The CD spectra and chromatography elution profiles of colicin E7 during one cycle of thermodenaturation: (**A**) Far-UV CD spectra of colicin E7 was reversible. Solid line, native curve of monmeric colicin E7 at 25°C; dotted line, denatural curve at 60°C ; and dashed line, renatural curve after recovery to 25°C. (**B**) Heat-treated colicin E7 was monitored by analytical size exclusion chromatography (Superose-12). Panel a, protein makers: Blue dextran 2000 (MW = 2,000 kDa) and Ovalbumin (MW = 43 kDa); panel b, monomeric colicin E7 maintains at 25°C in phosphate buffer; panel c, monomeric colicin E7 in phosphate buffer was denatured by heating to 60°C and renaturated by cooling to 25°C using the thermostated equipment.

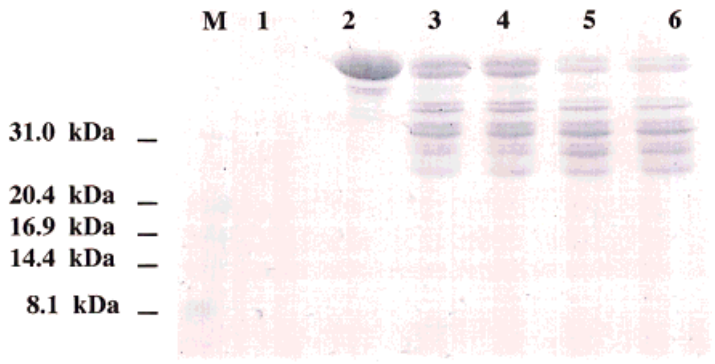

Fig. 9. Trypsin digestion profile of colicin E7: The detailed procedure is described in Materials and Methods. The digestion profiles were resolved by 10% SDS-PAGE. Lane 1, trypsin only; lane 2, colicin E7 only; lane 3 and 5, colicin E7 at low pH and digested with trypin for 10 and 20 min, respectively; lane 4 and 6, colicin E7 at neutral pH and digested with trypsin for 10 and 20 min, respectively; M: Markers. Sizes of molecular mass is shown.

slight structural change could induce a drastic change in the properties of colicin E7, the protein was allowed to slowly unfold by heating to 60°C and refolded by gentle recovery at room temperature. Temperature was strictly controlled and the CD spectrum was measured at the same time. The results shown in Figure 8A indicate that the unfolding-refolding processes of colicin E7 are reversible, as they display a similar spectrum pattern. Surprisingly, under this condition the monomeric colicin E7 converted to the multimeric form after the thermal unfolding cycle (Fig. 8B). The self-association of colicin E7 revealed that the aggregation-sensitive folding intermediates were produced during the refolding process. It is worth noting that the multimerization was detected when the dissociated colicin E7 was refolded by removing guanidine-HCl (Fig. 3).

There was no visible difference in trypsin digestion patterns of the protein at pH 2.5 and pH 8 (Fig. 9) indicating that a large decrease in Tm at low pH of the protein should not be caused by the conformational change of the protein. A compact denatured state of colicin E7 at low pH therefore may be either due to a small structural shift locally or a global loosening to a new structural state.

DISCUSSION

Three domains span the colicin E7 molecule and conduct the specific functions of translocation, receptor binding, and killing activity. The structural characteristics of these three domains are very distinct (Fig. 10). The receptor binding domain mainly contains α-helices and the translocation domain is composed of turns and sheets, whereas the nuclease domain is constructed with an equal number of helices and turns and/or sheets. The computer prediction for the secondary structure of colicin E7 coincides with data derived from CD spectrum (see Table I). The predicted α-helix content of colicin E7 (28% at pH 7.0) agrees with that of colicin E3 (30.7%),[17] even though their amino acid sequences are only 66.7% identical. In addition, the predicted secondary structure of colicin Ia (Fig. 10), a pore-forming colicin belonging to group B, agrees with the structure determined by X-ray crystallography,[24] indicating that the method for the prediction of secondary structure of colicins is reliable. It is worth noting that the receptor binding domain of colicin Ia is constructed by a unique β-sheet, while the corresponding domain of colicin E7 is formed mainly by α-helices. These results strongly suggest that translocation processes of the DNase- and ionophore-type colicins are different.

The monomeric form of colicin E7 is stable in physiological phosphate buffer. However, in the presence of some solvents, such as dimethyl sulfoxide and isopropanol, monomeric colicin E7 is precipitated as a multimeric form. This structural instability may result from oligomerization or multimerization which are induced in vitro by chemical and physical factors such as pH, solvents, and temperature. The trends of conformational change have been observed around the pore-forming colicin A,[26,27] and in the enzymatic colicin E3.[28,29] However, trypsin digestion of the aggregated colicin E7 at low pH (Fig. 9) shows no sign of conformational change, indicating that the structure of aggregated colicin E7 (DNase-type) is different from that of colicin A (pore-forming) and colicin E3 (RNase-type). Nevertheless, a large decrease in Tm of the protein at acidic pH (Fig. 7B) suggests that in membrane translocation phase it makes the protein much easier to unfold. In the absence of specific interacting targets at the aqueous phase in vitro, the total or partially unfolded colicins will refold into a uniform aggregative form (Fig. 8B). The aggregated colicins can only be dissociated by incubating the proteins with 6 N guanidine hydrochloride or heating with 50% formamide. These results clearly indicate that interactions between these aggregative forms of colicins are

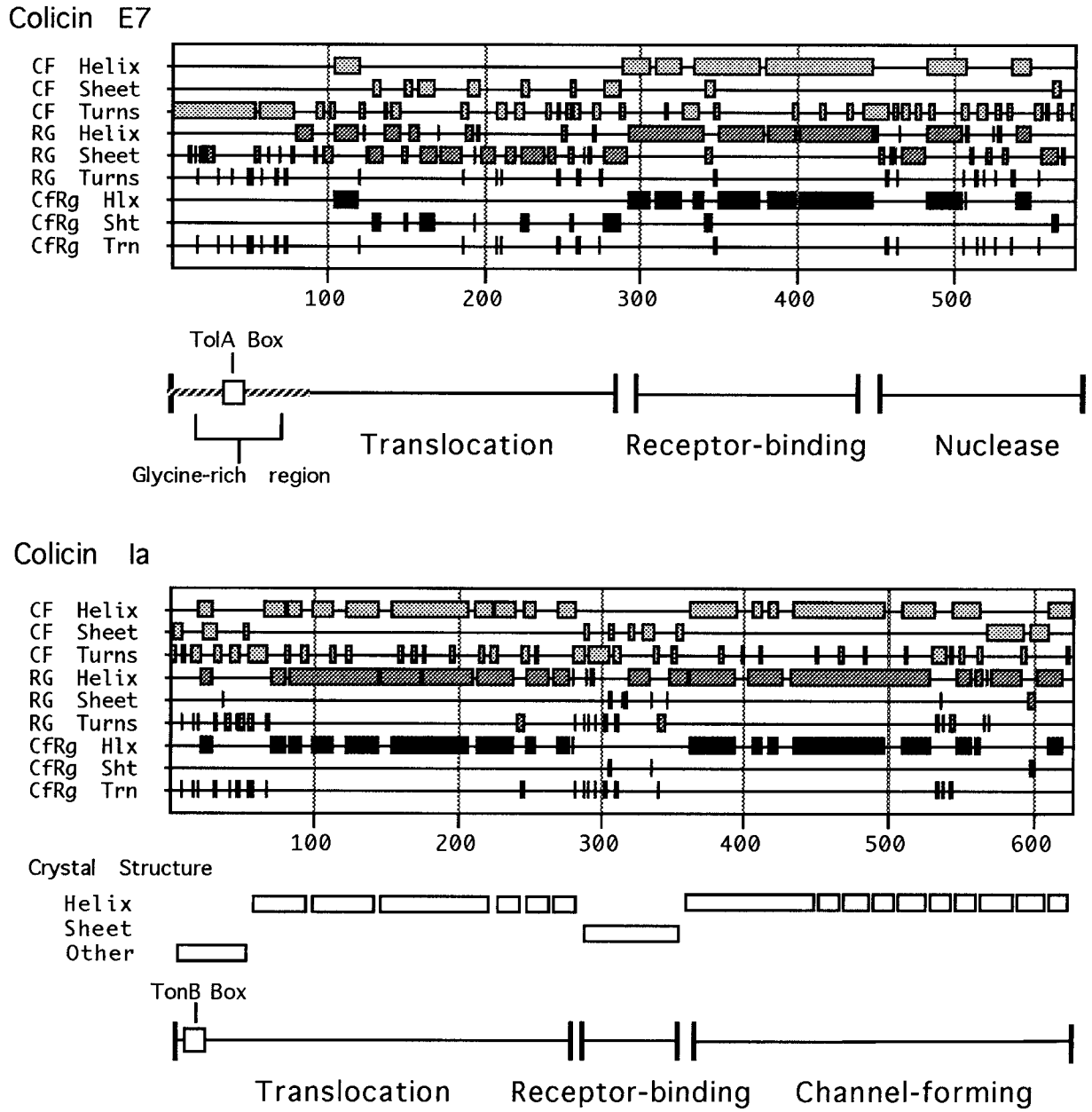

Fig. 10. Secondary structure predictions of colicins E7 and Ia: Enzymatic colicin E7 (group A) and pore-forming colicin Ia (group B) were analyzed based on their protein sequences. The figure illustrates the compositions of potential secondary structures of colicins according to the methods of Chou and Fasman[34] and Garnier et al.[35] The functional domains are indicated below the plots. The presentation of secondary structure of colicin Ia (solid box) deduced from the X-ray diffraction[24] is also shown. Symbols: CF: Chou-Fasman algorithm; RG: Robson-Garnier algorithm; CfRg: overlap of CF and RG. The plots were initially performed with MacVector programs (Eastman Chemical Company).

complicated. Probably, additional hydrogen bonds form between the molecules during the process of multimerization along with the hydrophobic interaction, as postulated in the association of diphtheria toxin multimers.[32] Multimers of colicin E7 exhibit in vivo bacteriocidal activity (Fig. 4), indicating that the protein is not aggregated nonspecifically and the retention of activity may suggest that there is some in vivo relevance. Perhaps, in vivo, interactions of colicin with specific targets such as receptor, chaperones, and phospholipids may favor the formation of the monomeric form and protect the unfolded colicins from being aggregated during translocation. Indeed, it was found that, in vivo, binding of Imm protein to its cognate colicin may play an important role in stabilizing the monomeric ColE3 from being aggregated.[28]

Recently, crystal structures of the monomeric and dimeric ImmE7 have been reported.[33] In this work,

formation of monomeric and dimeric ImmE7 has been further characterized. In normal physiological conditions, most of the ImmE7 are in dimeric form (Fig. 5A), while in the presence of guanidine hydrochloride they dissociate into monomeric form (Fig. 5B). We have proposed that the monomeric ImmE7 is a DNase-type inhibitor; but the dimeric ImmE7 is a novel specific RNase for the autoregulation of expression of the ColE7 operon.[33] Combining these two individual observations, we thus further predict that in vivo the monomeric ImmE7 must associate with its cognate colicin, whereas the excess ImmE7 must associate in dimeric form. Interchange of monomer and dimer of ImmE7 therefore play an important role in the regulation of the expression of the ColE7 operon. Investigation of monomer–dimer dynamic equilibrium of ImmE7 in vivo will be the major focus of our further studies.

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