Destabilization of the Colicin E9 Endonuclease Domain by Interaction with Negatively Charged Phospholipids

IMPLICATIONS FOR COLICIN TRANSLOCATION INTO BACTERIA*

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From the †Department of Biology, University of York, York YO10 5YW, United Kingdom, Schools of §Biological Sciences and ¶Chemical Sciences and Pharmacy, University of East Anglia, Norwich NR4 7TJ, United Kingdom, and ‡Institute of Infection, Immunity and Inflammation, Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom

We have shown previously that the 134-residue endonuclease domain of the bacterial cytotoxin colicin E9 (E9 DNase) forms channels in planar lipid bilayers (Mosbahî, K., Lemaître, C., Keeble, A. H., Mobasher, H., Morel, B., James, R. M., Moore, G. R., Lea, E. J., and Kleanthous, C. (2002) Nat. Struct. Biol. 9, 476–484). It was proposed that the E9 DNase mediates its own translocation across the cytoplasmic membrane and that the formation of ion channels is essential to this process. Here we describe changes to the structure and stability of the E9 DNase that accompany interaction of the protein with phospholipid vesicles. Formation of the protein-lipid complex at pH 7.5 resulted in a red-shift of the intrinsic protein fluorescence emission maximum (λmax) from 333 to 346 nm. At pH 4.0, where the E9 DNase lacks tertiary structure but retains secondary structure, DOPG induced a blue-shift in λmax from 354 to 342 nm. Changes in λmax were specific for anionic phospholipid vesicles at both pHs, suggesting electrostatics play a role in this association. The effects of phospholipid were negated by Im9 binding, the high affinity, acidic, exosite inhibitor protein, but not by zinc, which binds at the active site. Fluorescence-quenching experiments further demonstrated that similar protein-phospholipid complexes are formed regardless of whether the E9 DNase is initially in its native conformation. Consistent with these observations, chemical and thermal denaturation data as well as proteolytic susceptibility experiments showed that association with negatively charged phospholipids destabilize the E9 DNase. We suggest that formation of a destabilizing protein-lipid complex precedes channel formation by the E9 DNase and constitutes the initial step in its translocation across the Escherichia coli inner membrane.

Unraveling the interactions and mechanisms that enable proteins to cross biological membranes is of considerable interest, as the ability to target specific exogenous enzymes to the cytosol is likely to facilitate the design and discovery of novel chemotherapeutic agents. Many protein toxins have evolved to deliver a cytotoxic domain or subunit to the cytoplasm of susceptible cells, and so they provide an invaluable tool for studying protein translocation from the extracellular environment to their cellular targets, often located in the cytoplasm (1). The transition from the water-soluble to membrane-bound state has perhaps been most intensely studied in the pore-forming colicins (2, 3). This family of bacterial toxins, like all colicins, share a common three-domain structure, with receptor-binding and translocation domains that facilitate binding to the cell surface and mediate delivery of the channel-forming cytotoxic domain to the inner membrane. Cell death occurs as a consequence of ion channel formation across the cytoplasmic membrane, inducing depolarization of the membrane. Association of the cytotoxic domain with the membrane is thought to lead to destabilization and unfolding of the protein, yielding a “molten globule-like” state of loosely interacting helices (4, 5), from which a hydrophobic helical hairpin is able to spontaneously insert into the membrane (6, 7).

Electrostatic interactions are known to play an important role in mediating this interaction, particularly in the initial formation of the colicin-lipid complex (5, 8). The major phospholipid constituents of the Escherichia coli cytoplasmic membrane are phosphatidylethanolamine, phosphatidylglycerol (PG),1 and cardiolipin. The most abundant of these, phosphatidylethanolamine, is zwitterionic and usually accounts for around 70–80% of total cytoplasmic membrane phospholipid. Both PG and cardiolipin are anionic and account for the remaining 20–30% of phospholipid in an approximate 2:1 ratio (9). Both PG and cardiolipin are widely distributed throughout Gram-negative and Gram-positive bacteria and are, to a large degree, responsible for the overall anionic character of bacterial membranes.

Our current work focuses on the mechanism by which the cytotoxic DNase domain of the microbial toxin colicin E9 is able to translocate into the cytoplasm of susceptible cells to reach its cellular target, the bacterial chromosome. The E9 DNase domain shares no extensive sequence or structural similarities with the pore-forming colicins but does share with this group of toxins the ability to form ion channels in planar lipid bilayers (10). However, in contrast to the pore-forming colicins, which kill cells through depolarization of the inner membrane, the

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1 The abbreviations used are: PG, phosphatidylglycerol; DOPC, 1,2-dioleoyl-sn-glycero-3-phospho-rac(1-glycerol); DOPG, 1,2-dioleoyl-sn-glycero-3-phosphoglycerol; ANS, 8-anilinonaphthalene-1-sulfonic acid; ECP, eosinophil cationic protein.
channels formed by the E9 DNase domain do not in themselves cause cell death, because a mutant protein lacking DNase activity is still able to induce channel formation in planar lipid bilayers but is not cytotoxic (10). Instead, it was proposed that the observed channels are related to the ability of the E9 DNase domain to translocate across the inner membrane and must somehow reseal on entry of the domain into the cytoplasm. This possibility was inferred from the observation that the introduction of a specific disulfide bond in the E9 DNase domain had little effect upon the endonuclease activity of the DNase domain but abolished both channel activity and colicin cytotoxicity.

The 134-residue colicin E9 DNase domain is monomeric in solution (11) and, like all the enzymatic colicins, forms a high affinity complex with its cognate immunity protein, Im9 (12, 13). The immunity protein serves to protect the producing cell from the lethal effects of the toxin but must be jettisoned before translocation into the target cell. Unusually for an enzyme-inhibitor complex, the immunity protein does not bind directly to the active site of the E9 DNase, but rather to an adjacent exosite (14, 15). The catalytic center of the E9 DNase domain contains the HNH motif, which is the site for both DNA and metal binding (16). The HNH motif is also found in a variety of endonucleases, including the caspase-activated DNase that is responsible for degradation of the chromosome during eukaryotic apoptosis (16–18). The E9 DNase binds Zn\(^{2+}\) ions with nm affinity, and this interaction considerably stabilizes the protein (19).

However, for the DNase domain to be enzymatically active in vivo, Mg\(^{2+}\) ions are required, although these do not bind directly to the protein in the absence of DNA (16, 20). Intoxication of E. coli cells by colicin E9 induces the SOS response, the characteristic response to DNA damage, prior to cell death (21).

Here we describe the interaction of the E9 DNase with phospholipid vesicles. Working predominantly with anionic phospholipids, we studied the effects of the protein-lipid interaction on the structure and stability of the protein using fluorescence spectroscopy in combination with chemical and thermal denaturation experiments. We also compared the accessibility of the protein tryptophans to quenching agents with and without lipids. Our data show strong similarities to those resulting from the global changes that occur to the structure of pore-forming colicins during the initial stages of their association with negatively charged phospholipid membranes, and these are discussed in the paper.

**Materials and Methods**

**Protein Purification**—The E9 DNase domain and Im9 with a C-terminal 6-histidine tag were co-expressed from BL21 (DE3) cells containing the plasmid pRJ353 (22). The E9 DNase domain was purified by nickel-affinity chromatography, as described previously, with minor modifications (22). To ensure that the protein was metal-free, after nickel affinity chromatography, EDTA was added to a final concentration of 0 mM, and the protein was dialysed against 50 mM Tris-HCl, pH 7.2 and desalted by gel filtration chromatography in the same buffer (Superdex-75). The protein was then dialysed against 3 × 5 liters of 50 mM Tris-HCl, pH 7.5 containing 500 mM NaCl, 5 × 5 liters of 50 mM Tris-HCl, pH 7.5 containing 200 mM NaCl, 5 liters of 50 mM Tris-HCl, pH 7.5, and 3 × 5 liters of DI\(\H_2\)O. The protein was verified as being free of contamination by both metal and EDTA by its ability to bind a stoichiometric amount of zinc as determined by ANS binding, as described by Pommer et al. (19). The protein was then aliquoted, lyophilized, and stored at –20 °C. The concentration of the E9 DNase was determined from the absorbance at 280 nm using a molar extinction coefficient of 17,650 M\(^{-1}\) cm\(^{-1}\) (19). For experiments in which the zinc-bound protein was used, ZnCl\(_2\) was added to give a Zn\(^{2+}\)/E9 DNase ratio of 1:2.1.

**Lipid Vesicle Preparation**—1,2-dioleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (DOPG) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) were supplied by Avanti Polar Lipids (Alabaster, AL) and used without further purification. Lipid vesicles were prepared by using a film hydration method followed by extrusion through polycarbonate filters (23). Briefly, 2 mg of phospholipid in a 1:1 chloroform:methanol solution was dried by evaporation under nitrogen, and final traces of solvent were removed under vacuum. The lipid film was then hydrated with 1 ml of buffer and stirred at room temperature to form multilamellar vesicles. To form homogenous unilamellar lipid vesicles, the phospholipid solution was extruded 12 times through 0.2 µm polycarbonate filters. The presence of unilamellar vesicles was confirmed by electron microscopy after negative staining with ammonium molybdate (23).

**Fluorescence Measurements**—Fluorescence emission spectra were recorded on a Spex-FluroMax-3 spectrophotometer (Jobin Yvon) equipped with a Neslab RTE-111 circulating water bath. Spectra were recorded in 10 mM KPi, pH 7.5 at a protein concentration of 0.2 or 1 µM using an excitation wavelength of 280 nm, with excitation and emission slits set to 3 nm. Thermal denaturation profiles were obtained by monitoring the \(\lambda_{\text{max}}\) in the temperature range 15–80 °C. \(T_m\) values were obtained by fitting raw data to Eq. 1.

\[
y = y_o + a (1 + \exp(-x - x_o)) \quad (\text{Eq. 1})
\]

where \(y\) is the \(\lambda_{\text{max}}\), \(y_o\) is the starting value, \(x\) is the temperature, and \(x_o\) is the \(T_m\). Fluorescence emission was used to monitor the E9 DNase denaturation with increasing concentrations of urea. Solutions of the E9 DNase (1 µM) with urea at concentrations from 0–5 M were prepared in 50 mM Tris-HCl, pH 7.5 and incubated for 1 h at room temperature. Phospholipids in the form of lipid vesicles (DOPG and DOPC) were included at a protein to lipid ratio of 150:1, where shown. Quenching experiments using acrylamide were carried out by using an excitation wavelength of 295 nm at a protein concentration of 1 µM in either 50 mM Tris-HCl, pH 7.5, or 50 mM Tris acetate, pH 4.0, at a phospholipid:protein ratio of 150:1, where stated. Acrylamide, from a 5.63 M stock dissolved in water (Fluka Biochemika), was titrated into the protein or protein-lipid solution up to a final concentration of 0.2 M. All of the spectra were buffer-subtracted and corrected for dilution. The Stern-Volmer equation

\[
F_o/F = 1 + K_{sv}[Q] \quad \text{(Eq. 2)}
\]

was used to determine the Stern-Volmer quenching constant \(K_{sv}\), \(F_o\) and \(F\) are the fluorescence intensity in the absence and presence of the quencher (\(Q\)). Where this equation did not represent the data well, the modified Stern-Volmer equation (Eq. 3) was used to determine the accessibility of the tryptophan residues to the quencher.

\[
F_o/F = \frac{F_o}{F} = 1 + K_{sv}[Q] + 1/f_o \quad \text{(Eq. 3)}
\]

Therefore, a plot of \(F_o/F - F\) against 1/[\(Q\)] has a slope equal to 1/\(K_{sv}\) and an intercept equal to 1/\(f_o\). In this case, \(K_{sv}\) is the Stern-Volmer quenching constant for the accessible fraction of the tryptophan residues, and \(f_o\) is the fraction of the initial fluorescent, which is accessible to the quencher.

**Circular Dichroism**—Circular dichroism (CD) spectra of the E9 DNase were recorded on a Jasco J-810 spectropolarimeter equipped with a Jasco Peltier temperature controller (PFD-4255). Spectra were recorded in a 10-mm path length cuvette at a scan speed of 100 nm min\(^{-1}\), with a response time of 1 s and with the spectral bandwidth set to 1 nm. Measurements in the far-UV (190–300 nm) were recorded in 10 mM KPi, pH 7.5 at a protein concentration of 0.5 µM. The spectra obtained were the average of 10 scans with baseline subtraction.

**Proteolysis**—Tryptic digests of the E9 DNase domain were performed in 50 mM Tris-HCl, pH 7.5, at an E9 DNase concentration of 1 mg ml\(^{-1}\) and a trypsin concentration of 20 µg ml\(^{-1}\) at 37 °C. Zn\(^{2+}\) was present in slight molar excess (1:2:1) with DOPC or DOPG vesicles at 330 µM where indicated. Samples were removed at the times indicated, and proteolysis was stopped by the addition of an excess of trypsin inhibitor (Sigma). The products of proteolysis were analyzed by SDS-16% PAGE.

**RESULTS**

**E9 DNase Specifically Interacts with Negatively Charged Phospholipid Vesicles**—In aqueous solution close to neutral pH, the intrinsic fluorescence emission spectrum of the E9 DNase shows a maximum value (\(\lambda_{\text{max}}\)) of 333 nm, indicating that its two tryptophan residues are substantially buried in the interior of the protein. The intrinsic fluorescence of the E9 DNase is very sensitive to ligand-binding events, to the extent that binding at the immunity protein exosite and active site...
The shift in $\lambda_{\text{max}}$ was found to be approximately linearly dependent upon the molar lipid:protein ratio ($R_{L-P}$) with DOPG vesicles, up to an $R_{L-P}$ value of around 100, with the addition of further DOPG having little effect on the value of the $\lambda_{\text{max}}$ (Fig. 1B). Increasing the E9 DNase concentration 5-fold to 1 $\mu$M showed that the change in $\lambda_{\text{max}}$ is independent of the total lipid concentration and dependent only upon the lipid:protein ratio (data not shown). With neutral DOPC vesicles, no change in $\lambda_{\text{max}}$ was observed up to a lipid:protein ratio of 500, indicating a strong electrostatic contribution to the protein-lipid interaction with DOPG. Using mixed phospholipid vesicles consisting of equimolar amounts of DOPC and DOPG, we observed a similar dependence of the $\lambda_{\text{max}}$ upon the value of $R_{L-P}$ with DOPG alone, but with a reduced effect upon the total change of the $\lambda_{\text{max}}$ (Fig. 1B).

Probing the Structure of the Protein-lipid Complex by Fluorescence Quenching—At pH 4.0, near-UV CD indicates that the E9 DNase does not possess a well defined tertiary structure, whereas far-UV CD indicates only subtle changes in secondary structure compared with the protein at neutral pH (24). The absence of persistent tertiary structure for the E9 DNase at pH 4.0 is confirmed by fluorescence spectroscopy, where $\lambda_{\text{max}}$ is red-shifted from 333 to 354 nm, indicating complete solvent exposure of the two tryptophans (Fig. 1A). Upon the addition of DOPG phospholipids at pH 4.0 ($R_{L-P} = 150$), we found that there was a significant blue-shift to $\sim 341$ nm, but there was no shift in the $\lambda_{\text{max}}$ upon addition of DOPC (data not shown). Not only does this illustrate that complexation between the E9 DNase and phospholipids does not require defined tertiary structure, but it implies that electrostatics are the likely basis for this association. We return to this issue in “Discussion.”

The $\lambda_{\text{max}}$ in the presence of DOPG at pH 4.0 is similar to that observed upon addition of DOPG to the E9 DNase at pH 7.5 (Fig. 1A), suggesting that the solvent accessibility of the tryptophans in the final protein-lipid complex is similar at both pHs. This is supported by fluorescence-quenching experiments in the presence and absence of DOPG vesicles with the water-soluble quencher acrylamide at pH 4.0 and 7.5. Fig. 2 shows Stern-Volmer plots for the E9 DNase under these conditions. In the case of E9 DNase in solution at pH 4.0, the Stern-Volmer plot displayed a downward curvature (Fig. 2A); therefore, a modified Stern-Volmer plot was used (Fig. 2B). As expected, the accessibility of the E9 DNase tryptophan residues to acrylamide at pH 4.0 ($K_{sv} = 11 \text{ M}^{-1}$), where the protein is unfolded, is much greater than in the folded state at pH 7.5 ($K_{sv} = 2.7 \pm 0.1 \text{ M}^{-1}$). However, in the presence of DOPG vesicles at pH 4.0, the accessibility to acrylamide decreases to a value similar to that of the folded protein ($K_{sv} = 2.3 \pm 0.1 \text{ M}^{-1}$) and is very similar to that of the E9 DNase in the presence of DOPG vesicles at pH 7.5 ($K_{sv} = 2.1 \pm 0.1 \text{ M}^{-1}$). These data are consistent with the E9 DNase forming non-voltage gated channels in planar lipid bilayers both at pH 4.0 and pH 7.5 and indicates that the protein does not have to be in its native conformation to interact with membranes.2

Effect of Ligand Binding and Disulfide Bond Formation on the Ability of the E9 DNase to Interact with Lipids—The experiments described above were performed on protein preparations of the E9 DNase in the absence of bound metal. However, it has been shown previously that binding of a stoichiometric amount of Zn$^{2+}$ causes a considerable increase in the conformational stability of the E9 DNase (19). This is manifest by an increase of 22 °C (from 37 to 59 °C) in the melting temperature of the protein at pH 7.5, a considerable decrease in the susceptibility of the protein to proteolysis, and a decrease in affinity of

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2 K. Mosbah and C. Kleanthous, unpublished results.
the protein for the hydrophobic dye ANS (19). In view of these differences, we performed a similar experiment to that described in Fig. 1B in the presence of zinc (Fig. 3A). We found no difference in the change in $\frac{\lambda_{\text{max}}}{H}$ between the zinc-bound form and the metal-free protein upon association with DOPG vesicles, showing that the ability of the E9 DNase to bind negatively charged phospholipids is not affected by the conformational stability of the protein. This result is also consistent with the observation that the presence of bound zinc has no effect upon the ability of the E9 DNase to form channels in planar lipid bilayers (10). The E9 DNase forms a high affinity complex with its cognate immunity protein Im9 (12). The formation of this heterodimeric complex has been shown previously to prevent channel formation by the E9 DNase in planar lipid bilayers (10). Consistent with this, we observed no change in the $\lambda_{\text{max}}$ of the E9 DNase-Im9 complex ($\sim 330$ nm) upon addition of DOPG vesicles at an $\text{R}_{L/P} = 150$ (Fig. 3B). Therefore, we infer that the presence of the immunity protein prevents the formation of a protein-lipid complex.

We have shown previously that the ability of the DNase to form channels in planar lipid bilayers can be abolished by the formation of an artificial intramolecular disulfide bond between residues 20 and 66 (10). This loss of channel activity is accompanied by a loss of cytotoxicity for the intact toxin, but there is little effect upon its endonuclease activity. Therefore, the formation of this disulfide bond affects the ability of the E9 DNase to translocate across E. coli cellular membranes. Because this disulfide-containing mutant of the E9 DNase differs from the wild-type protein in its ability to form channels in planar lipid bilayers, we tested its ability to interact with DOPG vesicles using fluorescence. Monitoring the change in the $\lambda_{\text{max}}$ at an increasing lipid-protein ratio (as in Fig. 1B) gave an essentially identical profile to that observed for the wild-type protein (data not shown). Thus, the ability of the E9 DNase to interact with phospholipid vesicles, as monitored in this work, is not on its own sufficient for the protein to form ion channels in planar lipid bilayers.

**Fig. 2.** Association of the E9 DNase with DOPG vesicles leads to protection from the quencher acrylamide and is pH independent. A, Stern-Volmer plots for the E9 DNase in the presence and absence of DOPG vesicles at pH 7.5 and pH 4.0. $F_0$ is the fluorescence in the absence of acrylamide. Aliquots of acrylamide were titrated into the protein (1 μM) or into the protein-lipid mixture at 25 °C. B, modified Stern-Volmer plot of the two populations of tryptophan residues of the E9 DNase at pH 4.0 in the absence of phospholipid vesicles, with $1/f_0$ as the intercept and $1/f_0K_a$ as the slope (see “Materials and Methods” for details).

**Fig. 3.** Effect of ligand binding on E9 DNase interactions with phospholipid vesicles. A, changes in the intrinsic fluorescence emission maxima ($\lambda_{\text{max}}$) of the E9 DNase on lipid:protein molar ratio, in the presence and absence of zinc bound to the protein. Conditions are the same as for Fig. 1B. Experiments were performed in 10 mM KPi, pH 7.5 at 25 °C. B, intrinsic fluorescence emission spectra of E9 DNase in complex with its cognate immunity protein Im9, in the absence and presence of DOPG vesicles. Experiments were performed at a protein concentration of 1 μM and a phospholipid concentration of 150 μM ($\text{R}_{L/P} = 150$) in 10 mM KPi, pH 7.5 at 25 °C.
The DNase domain of colicin E9 must traverse both the outer and inner membranes of sensitive *E. coli* cells to reach its...
negatively charged phospholipid vesicles causes secondary structure of the E9 DNase. In the absence and in the presence of DOPC and DOPG vesicles at a concentration seem to be needed to diminish the DOPG-influences pH 4.0 to form a protein-lipid complex with a similar protein E9 DNase can readily interact with DOPG vesicles at pH 7.5 or of secondary structure at pH 4.0 but little or no tertiary structure where interactions between secondary structural elements in the native state are replaced by interactions with the lipid bilayer in the membrane-bound state. A similar situation is thought to occur in the initial formation of the protein-lipid complex of the cytotoxic domains of the pore-forming colicins, such as colicin A and E1, with anionic phospholipids (5, 8).

The electrostatic contribution to binding between phospholipids and the E9 DNase can be inferred from the lack of a significant effect upon the interaction with zwitterionic DOPC vesicles and the reduction of this destabilizing effect for mixed DOPC and DOPG vesicles relative to those containing DOPC alone. Moreover, preliminary analysis of the effect of increasing salt is also consistent with electrostatics playing an important role. At pH 4.0 and 7.5, the DOPG-induced changes to the E9 DNase fluorescence $\lambda_{\text{max}}$ are significantly diminished. For example, at pH 7.5, 200 mM NaCl is sufficient to completely abolish the DOPG-induced red-shift. At pH 4.0, higher salt concentrations seem to be needed to diminish the DOPG-induced effect on $\lambda_{\text{max}}$, presumably reflecting the increase in overall positive charge of the E9 DNase at this pH. Finally, binding of the immunity protein, Im9, abolishes protein-lipid complex formation. We speculate that this is probably because of decreasing the electrostatic interaction with anionic phospholipids upon binding of the acidic immunity protein (pI = 4.5) to the exosite of the basic E9 DNase domain (pI = 9.5).

The importance of electrostatic interactions in nucleosome in the absence or presence of DOPC and DOPG vesicles, which was detected by Western-blot assay. It is thought that the basic nature of the protein may help it to bind to the negatively charged polar head group and induce an ion channel across the membrane that is thought to be the origin of its cytoxicity (29). This is in contrast to the E9 DNase that kills cells through a nonspecific endonuclease activity (16).

We have shown previously that the formation of a disulphide bond D20/C66C abolishes both cytotoxicity and channel-forming activity of the E9 DNase without affecting its endonuclease activity (10). The same disulfided protein was still capable of binding to DOPG phospholipid vesicles, showing that channel-formation is a distinct step in the association of the E9 DNase with phospholipids. A similar effect has been observed previously with the pore-forming domain of colicin A, in which disulfided mutant proteins that were not able to form ion channels retained the ability to insert into phospholipid vesicles in a manner similar to the wild-type protein (32). Thus, as for the pore-forming colicins, there seem to be distinct uncoupled steps in membrane interaction and protein translocation for the E9 DNase.

At the present time, aspects of the interaction of the E9 DNase with anionic phospholipid vesicles are unclear. For example, it is difficult to certain about the extent to which the E9 DNase penetrates the membrane, or indeed, if the protein interacts only transiently with the vesicle surface. In addition, the nature of the changes to E9 DNase secondary structure is unclear, making it difficult to formulate a mechanism for its translocation. A model for the mechanism of translocation of antimicrobial cationic peptides, such as magainin-2, gramicidin S, cecropin, and melitin, has been proposed by Matsusaki (33). It has been suggested that the peptide binds initially to the membrane surface by electrostatic interactions with the polar head group of the lipids. Thinning of lipid bilayer takes place after the spontaneous insertion of the antimicrobial peptide, inducing the formation of transient pores that cause disruption of the membrane, displacement of lipids, and complete entry of the peptide into the target cell (34).

We suggest that the changes to the secondary and tertiary structure of the E9 DNase described here represent the initial stage in the protein-lipid interaction that ultimately results in translocation of the E9 DNase across the membrane. Comparison of our data upon the E9 DNase with related studies upon the pore-forming colicins suggests that this initial stage in the protein-lipid interaction may be similar in both cases. However, in the latter case, the ultimate end of this process is the formation of voltage-gated channels and depolarization of the membrane, whereas in the case of the E9 DNase, it is the
translocation of the entire protein across the membrane into the cytoplasm.

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