The Central Domain of Colicin N Possesses the Receptor Recognition Site but Not the Binding Affinity of the Whole Toxin†

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ABSTRACT: Colicin N is a three-domain pore-forming colicin which kills enterobacterial cells following an initial binding to its receptor, the outer membrane porin OmpF. The receptor-binding domain of colicin N alone, and attached to the translocation domain, was overexpressed and purified using a hexahistidine tag. The receptor domain attached to the pore-forming domain was obtained by enzymatic digestion. Circular dichroism spectroscopy showed that the domains have structure in keeping with the known structure of colicin N. The receptor domain was stable, retaining both secondary and tertiary structure in 2 M guanidine hydrochloride and at low pH. It bound to both OmpF and PhoE porin-producing *Escherichia coli* with no toxicity and protected sensitive *E. coli* against intact colicin N toxicity at high domain/colicin N ratios. Its *in vitro* affinity for OmpF, as determined by isothermal titration microcalorimetry, was found to be approximately 50-fold weaker than that of native colicin N. The receptor domain was readily out-competed by native colicin N in *in vivo* fluorescence assays which, coupled with its structural stability, suggests that its interaction with OmpF is one of weak, reversible binding. Since neither of the double domain constructs shows wild-type binding affinity either, it appears that the molecular recognition is a property of the receptor domain but that affinity is influenced by the entire molecule.

The physical biochemistry of protein import and export from cells is poorly understood apart from the feature that some systems [i.e., mitochondrial protein import (Lithgow *et al.,* 1995)] clearly require the protein to adopt a non-native configuration. In this context, we have been using colicin N to study protein import across the *Escherichia coli* outer membrane. This system is of great utility since it is very simple and structurally well characterized. It involves a single, abundant receptor, OmpF, and the smallest known colicin. The three-dimensional structures of OmpF (Cowan *et al.,* 1992) and residues 66−387 of colicin N[1] are known to high resolution by X-ray crystallography.

Colicins are plasmid-encoded bacteriocins produced by *E. coli* which kill sensitive *E. coli*. The toxic action is known to occur in three distinct stages: (1) receptor recognition and binding; (2) translocation across the cell envelope; (3) lethal action in the form of pore-formation in the cytoplasmic membrane, DNase or RNase activity (Benedetti *et al.,* 1992; Cramer *et al.,* 1983; El-Kouhen *et al.,* 1993). Specific domains of colicins have been assigned to each stage of interaction [for a review, see Cramer *et al*. (1995)].

Colicin N is a pore-former belonging to the A group of colicins (Davies & Reeves, 1975) (A, E_{1-9}, K, and N) which require the tol QRAB proteins for translocation across the membrane (Webster, 1991). Colicin N requires the receptor OmpF for full activity (Pugsley, 1984, 1987; Tommassen *et al.,* 1984) *in vivo*, and the related porins OmpC and PhoE are associated with greatly reduced cellular sensitivities to colicin N (Bourdineaud *et al.,* 1990; Tommassen *et al.,* 1984). However, we have recently shown that *in vitro* colicin N can bind to all three porins with similar affinity (Evans *et al.,* 1996).

Little is known about the mechanism of colicin−receptor interaction and its subsequent translocation. It appears to follow the general rule (for import at least) that significant unfolding should occur prior to or during translocation (Benedetti *et al.,* 1992). An OmpF mutant, G119D, selected on the basis of its resistance to colicin N, has its mutation on a peptide loop within the pore, suggesting either that the colicin N binding site is deep inside the pore or that colicin N translocates through the pore (Jeanteur *et al.,* 1994). Studies on mutants of colicin A C-terminal fragment indicate that colicin A unfolds on binding to its receptor (Duché *et al.,* 1994), and calorimetric measurements on colicin N show that it undergoes exothermic structural rearrangements on binding to OmpF (Evans *et al.,* 1996).

We are interested in elucidating the colicin N−receptor interaction and receptor function, and determining why OmpF is the preferred receptor *in vivo*. The three domains of colicin N have been defined previously using proteolytic fragments, truncated at the N-terminus, in *in vivo* binding and cell survival assays (El-Kouhen *et al.,* 1993). The receptor domain has been identified as residues 67−182, the translocation domain as residues 1−66, and the pore-forming domain as residues 183−387. We have therefore subcloned and expressed residues 1−190 (TR) and 67−190 (R) to see whether these form stable and functional domains. Together

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with the chymotryptic fragment [receptor and pore-forming domains (El-Kouhen *et al.*, 1993), RP], we have characterized the *in vivo* and *in vitro* interactions of the receptor domain of colicin N with OmpF.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids. A modified pET8c vector (Politou *et al.*, 1994) with *Xho*I and *Mlu*I restriction sites was used to overexpress the domains. The vector introduces a methionine, six histidines, and two serine linkers at the N-terminus of the inserted gene, which is under the control of a T7 promoter. Inserts were prepared by PCR using the template pChap4 (Pugsley, 1987) which encodes for wild-type colicin N, and primers with 18 bp matching sequence and restriction sites for *Xho*I and *Mlu*I. The receptor domain, R, corresponded to bases 814−1186 and the receptor plus translocation domains, TR, to 616−1186 of the *cna* structural gene (Pugsley, 1987). The PCR products were gel purified and ligated into purified, digested vector. The product was used initially to transform *E. coli* JM103 cells, with ampicillin selection. Successful transformants were selected on the basis of miniprep restriction digest analysis or analytical PCR. A subsequent plasmid preparation was used for DNA sequencing and to transform *E. coli* BL21(DE3).

Protein Purification. E. coli BL21(DE3) containing the plasmids for both R and TR were grown in L-broth (Sambrook *et al.*, 1989) containing ampicillin (100 μg/mL). Following induction at $OD_{650} = 0.7$ with isopropyl β-D-thiogalactopyranoside IPTG[2] at 1 mM, the bacteria were grown for a further 5 h. The cells were harvested and resuspended in 20 mM phosphate, 0.7 M NaCl, pH 7, containing RNase A (20 μg/mL) and DNase (20 μg/mL). Cells were lysed by sonication, and the supernatant was obtained by centrifugation at 100000*g* for 1 h. Both proteins R and TR were found to be soluble with none remaining in the cell pellet. The proteins were purified by use of the 6 × His tag on a Qiagen Ni-NTA affinity column (Hochuli *et al.*, 1988). The proteins were washed onto the columns with 50 mM phosphate, 300 mM NaCl, pH 8 buffer, and eluted in 250 mM imidazole, pH 7. Further purification was carried out on a S-Sepharose (Pharmacia) cation exchange column, equilibrated with 20 mM phosphate, 300 mM NaCl, pH 5, using a NaCl gradient to 1 M NaCl. R and TR both elute at approximately 0.7 M NaCl. The proteins were analyzed for purity by SDS−PAGE. The M_r of each recombinant domain was confirmed by MALDI-TOF mass spectrometry (Kratos, Manchester, U.K.).

Colicin N was purified from *E. coli BZB1019* (hsdR) *carrying* pChap4 (Pugsley, 1987), as previously described (El-Kouhen *et al.*, 1993). The α-chymotryptic fragment was prepared from colicin N as previously described (El-Kouhen *et al.*, 1993). OmpF was purified from *E. coli* B^E3000 (Garavito & Rosenbusch, 1986) as previously described (Lakey *et al.*, 1985). All protein concentrations were determined by UV absorption at 280 nm.

[2] Abbreviations: ANS, 8-anilino-1-naphthalenesulfonic acid; BSA, bovine serum albumin; CD, circular dichroism; Gdn·HCl, guanidine hydrochloride; IPTG, isopropyl β-D-thiogalactopyranoside; ITC, isothermal titration calorimetry; LB, Luria−Bertani broth; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; UV, ultraviolet.

Spectroscopy. Far-UV CD spectroscopy was carried out on a Jobin Yvon CD6 spectrophotometer. Measurements were made using a 0.01 cm path length circular cuvette (Hellma), protein concentrations of 0.7−2.0 mg/mL, and a bandwidth of 2 nm. Proteins were in 10 mM phosphate, 300 mM NaCl, pH 6.8, buffer, and measurements were carried out at 25 °C.

For the acid unfolding of R, a stock solution of the protein was diluted into buffers of 10 mM phosphate, 300 mM NaCl, and either citric acid, pH 6.0−2.0, or Gdn·HCl, pH 6, and equilibrated for 1 h, room temperature. Near-UV CD measurements were made using a 1 cm path length cuvette (Hellma) at 25 °C. For unfolding experiments, stock solutions of R were diluted into 200 mM phosphate, pH 6.5, containing Gdn·HCl (0−6 M) and allowed to equilibrate for 1 h, room temperature, prior to collection of far-UV spectra as above. The percentage of unfolding was calculated assuming that R was fully folded at 0 M Gdn·HCl, and fully unfolded at 6 M Gdn·HCl.

In Vivo Binding Assays. E. coli strains expressing only OmpF, B^E3000 (Garavito & Rosenbusch, 1986); only PhoE, CE1197 (Tommassen *et al.*, 1984); only OmpC, RAM105 (Misra & Benson, 1988); and no porins at all, ECB611 (Misra & Benson, 1988), were grown in L-broth to an OD_{600} of 0.5 (5×10^8 cells/mL). The cells were harvested and then resuspended in 20 mM phosphate, 300 mM NaCl, pH 7, to a concentration of 5×10^{10} cells/mL. Eight microliters of cells was mixed with 20 μL of protein solution. Wild-type colicins N, R, and TR and a control of BSA were used at concentrations of 0.3−0.4 mg/mL. Following incubation at room temperature for 20 min, the cells were pelleted and the supernatants analyzed for the added protein by SDS-PAGE.

Cell Survival Assays. Cell survival assays have been previously described (Bourdineaud *et al.*, 1990; Cavard, 1994; El-Kouhen *et al.*, 1994; Fourel *et al.*, 1990). B^E3000 cells grown in L-broth to an OD_{600nm} of 0.5 were incubated 1:1 with dilutions of R in 10 mM phosphate, 300 mM NaCl, pH 7.4, or just buffer for 30 min at room temperature;100 μL of this suspension was added to 100 μL of 10-fold dilutions of colicin N (stock 200 μg/mL) in L-broth and incubated for 15 min at 37 °C; 1.8 mL of fresh LB was added, and the cells were grown at 37 °C for 2 h. Percentage survival was determined by the ratio of OD_{600nm} of colicin N-treated to untreated cells. Protection was evaluated by comparison of R-treated and untreated cells.

In Vivo Fluorescence Assay. Cell sensitivity assays using fluorescent probes have been previously described (Brunden *et al.*, 1984; Cramer & Philips, 1970). B^E3000 cells were grown in L-broth to an OD_{600} of 0.5 (5×10^8 cells/mL), washed in water, reconstituted in 10 mM phosphate, pH 7.4, to a concentration of 5×10^9cells/mL, and stored on ice. Measurements were made on an Aminco SLM 8100 spectrofluorometer at 25 °C with a 1 cm pathlength cuvette (Hellma). For each measurement, 0.3 mL of cells were mixed with 2.7 mL of phosphate buffer, and 150 μL of ANS was added (1 μM stock) and left to equilibrate. Five microliters of R domain at various concentrations (see figure legends) was added after 600 s, with 2.5 μL of colicin N, 10 μg/mL added at 1000 s. The fluorescence increase was monitored for 45 min.

Isothermal Titration Calorimetry. ITC was carried out at 25 °C following standard procedures (Cooper & Johnson,

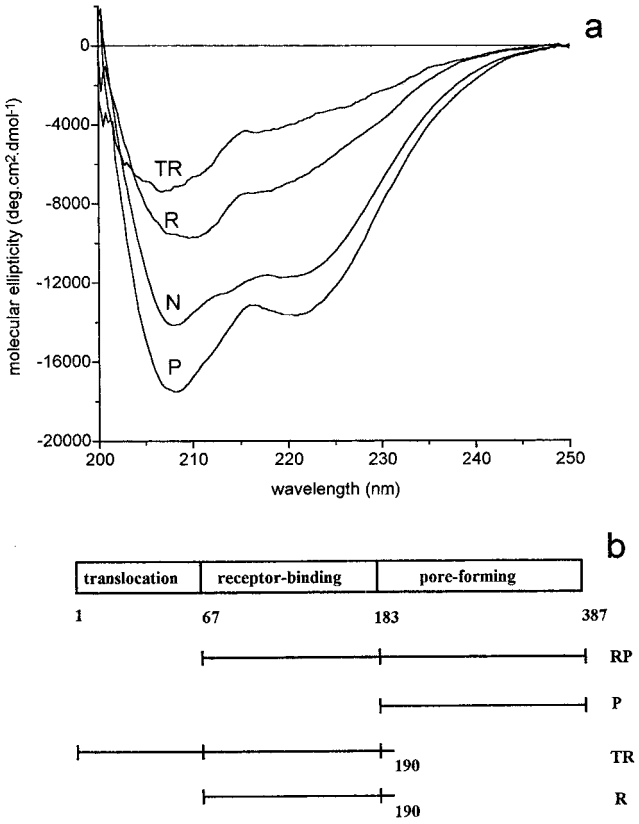

FIGURE 1: Far-UV CD spectra of native colicin N, pore-forming fragment, and cloned domains. (a) A comparison of the secondary structures of the cloned domains with native colicin N was made at 25 °C using a circular cuvette of 0.01 cm path length and protein concentrations of 0.7−2.0 mg/mL. TR, cloned translocator plus receptor domains; R, cloned receptor domain; N, native colicin N; P, thermolytically produced pore-forming fragment. (b) Domain structure of colicin N (El-Kouhen *et al.,* 1993).

1994; Wiseman *et al.,* 1989) with an Omega titration calorimeter (MicroCal, Northampton, MA), using a 250 μL injection syringe with 400 rpm stirring. Proteins (see figure legends for concentrations) were dialyzed into 20 mM Tris, 300 mM NaCl, 1% octyl-POE prior to ITC measurements, and the dialysis buffer was used for protein heat of dilution control experiments. Calorimetric data were analyzed using MicroCal Origin software.

RESULTS

The Receptor Domain of Colicin N Is Stable and Retains Its OmpF-Binding Site. Colicin N residues 1−190 (TR) and 67−190 (R) were successfully cloned and purified at yields of 5 mg/L of culture. The domains were stable, and mass spectra gave the expected molecular weights of 21 799 ± 22 and 14 797 ± 15, respectively. Figure 1 compares the far-UV CD spectra of TR, R, native colicin (N), and the pore-forming domain (P). The high-resolution structure has been solved[1] for colicin N residues 90−383; the pore-forming domain is a 10 α-helical bundle, homologous to colicin A pore-forming fragment (Parker *et al.,* 1989, 1992), and the section of R solved is β-sheet-folded around a central helix. The decrease in molecular ellipticity observed (P > colicin N > R) was therefore in keeping with the decreasing proportion of helix in the known structures. The translocation domain (residues 1−66) has a glycine/proline/serine/asparagine-rich sequence which we would expect to have a poorly defined secondary structure and would account for the weaker signal of the TR domain.

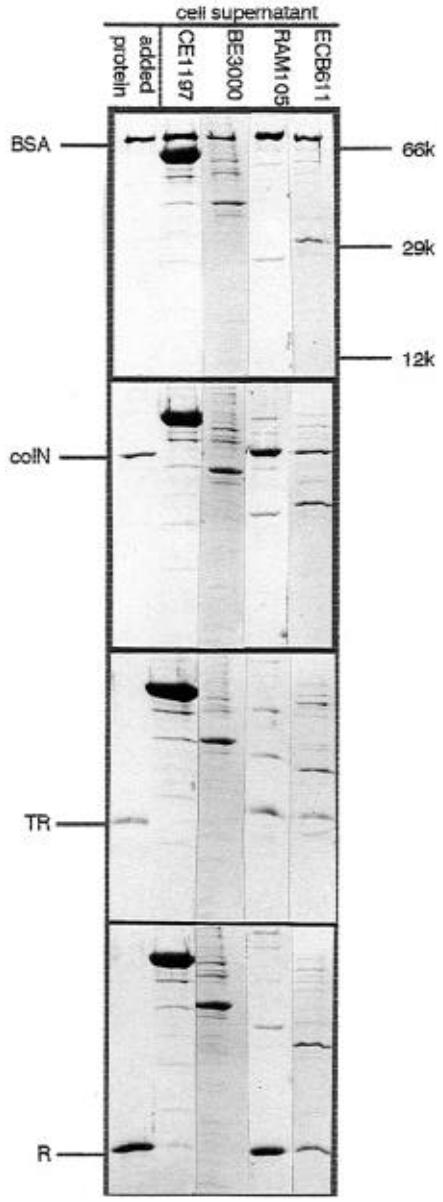

FIGURE 2: *In vivo* binding of cloned domains. Colicin N and the cloned domains were individually incubated with suspensions of four non-porin/porin-producing strains. Following centrifugation, the supernatants were analyzed by SDS−PAGE for protein. (a) BSA, added at 0.3 mg/mL; (b) colicin N, added at 0.4 mg/mL; (c) TR, added at 0.3 mg/mL; (d) R, added at 0.4 mg/mL. Lane 1, pure protein, used as marker; lanes 2−5, proteins remaining in supernatant of CE1197, B^E3000, RAM105, and ECB611, respectively, following pelleting of cells. (The extra bands seen in these lanes reflect proteins released from the cells during incubation with 300 mM NaCl buffer.)

The *in vivo* binding ability of R and TR was determined by their removal from solution by OmpF-expressing cells as shown (Figure 2). Strains producing the homologous (but nonreceptor) porins OmpC and PhoE were also screened. R (2d) and TR (2c) bound to the OmpF and PhoE producing strains B^E3000 and CE1197, but not the nonporin strain ECB611 or the OmpC strain RAM105. Colicin N (2b) was also unable to bind to RAM105. Since binding of colicin N to OmpC occurs *in vitro* (Evans *et al.,* 1996a) this may reflect low accessibility of the porin-binding site to colicin N within this strain. A control of BSA (2a) did not bind to

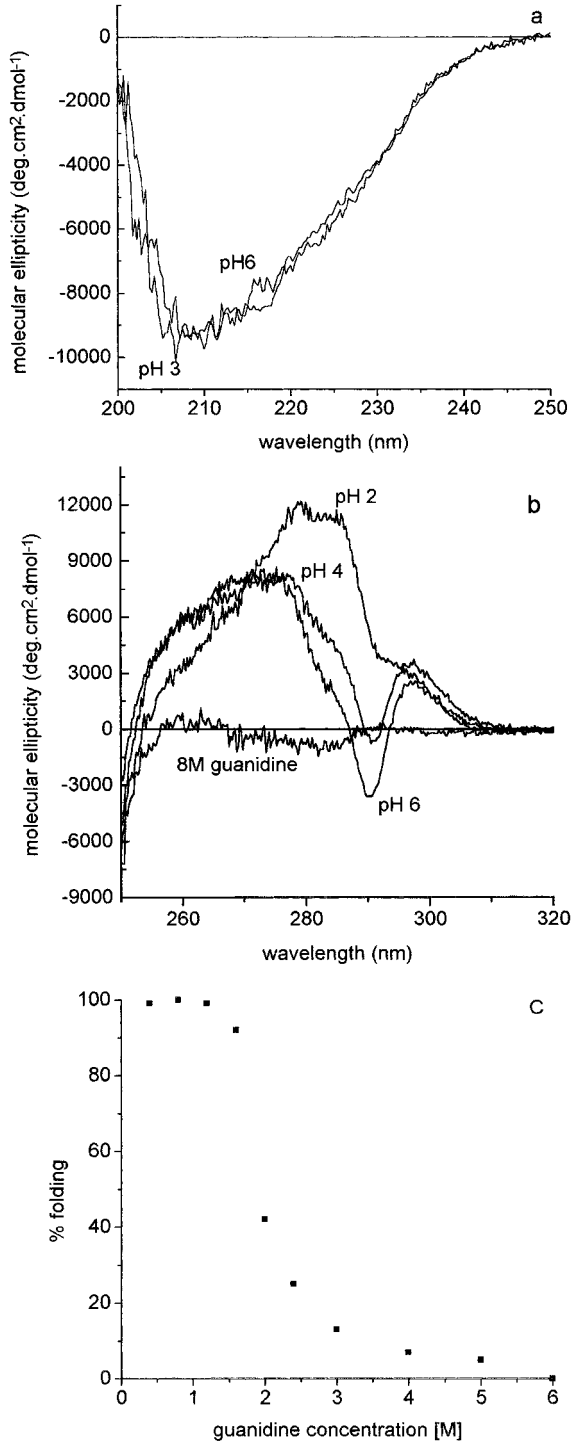

FIGURE 3: Acid and Gdn denaturation of the colicin N receptor domain. A stock solution of R was diluted into 20 mM phosphate/citrate, 300 mM NaCl buffer, pH 2−6; or 200 mM phosphate/Gdn (0−6 M), pH 6.5, and allowed to equilibrate for 1 h prior to data collection at 25 °C. (a)Far-UV CD spectra, pH 3−6. Data were collected using a circular cuvette of 0.01 cm path length and protein concentrations of 0.5 mg/mL. (b) Near-UV CD spectra, pH 2−6. Data were collected using a 1 cm path length and protein concentrations of 0.5 mg/mL. (c) Gdn unfolding curve as determined by far-UV CD; concentrations and cuvettes as in (a).

any porin strain.

The Receptor Domain of Colicin N Remains Folded at Acid pH. The secondary and tertiary structure of R at acidic pH was determined by circular dichroism spectroscopy. The secondary structure of R showed little change between pH 3 and 6 (Figure 3a), remaining as a $\alpha+\beta$ structure. The tertiary structure showed slight changes (Figure 3b) with the peak at 280 nm arising from the single tryptophan in R decreasing as the pH is lowered. However, compared to the unfolded structure of R in 6 M Gdn, most of the tertiary structure is retained, which is in contrast to the pore-forming domain of colicins A (Muga *et al.*, 1993; van der Goot *et al.*, 1992), E_1 (Schendel & Cramer, 1994), and Ia (Mel & Stroud, 1993), which lose tertiary structure at low pH. The Gdn·HCl unfolding of R (Figure 3c) showed cooperative unfolding with a single transition at 2 M Gdn, indicative of a folded, single domain.

The Receptor Domain Confers Limited Protection against Colicin N. It has been shown for colicins A and E_1, which use BtuB (vitamin B_{12} receptor) as their outer membrane receptor, that preincubation of sensitive cells with vitamin B_{12} can protect against colicin action in cell survival assays (Cavard, 1994; Cavard & Lazdunski, 1981). For colicins A and N, monoclonal antibodies directed toward OmpF also gave protection (Bourdineaud *et al.*, 1990; Pagès *et al.*, 1988). Presumably, in both cases this is because the receptor-binding site is blocked. We therefore tried to protect B^E3000 cells from colicin N toxicity by blocking the receptor sites with R. Figure 4a shows the effect of preincubation with R in cell survival assays. Preincubating the sensitive *E. coli* with R at 12.5 μM [a multiplicity of 10^7/cell (Bourdineaud *et al.*, 1990; El-Kouhen *et al.*, 1993; Fourel *et al.*, 1990)] gave 2-fold and 5-fold increases in cell survival at multiplicities of 100 and 1000 colicin N molecules/cell, respectively, but had little effect at higher multiplicities of colicin N. With lower concentrations of R, virtually no protection was observed (data not shown). This protection compares poorly to vitamin B_{12} which protects against colicins A and E significantly at 1 μM (Cavard, 1994), and to MoF18 antibodies directed against OmpF which significantly protected *E. coli* B cells against colicin N at a multiplicity of $(3-5) \times 10^5$/cell (Bourdineaud *et al.*, 1990).

As a second test for R domain protection against colicin attack, we used an ANS assay (Cramer & Philips, 1970). The addition of colicin to sensitive cells equilibrated with surface-bound self-quenching ANS can cause an increase in fluorescence as the colicin pore depolarizes the cytoplasmic membrane and the ANS redistributes more widely within the cell membrane. The effect of adding increasing amounts of R domain to B^E3000 prior to the addition of 25 ng of colicin N to the cuvette (a multiplicity of 100/cell) is shown in Figure 4b. R significantly slows the rate of fluorescence increase, even at 50 ng (a multiplicity of 600/cell). R, however, cannot totally prevent colicin N action as maximum fluorescence is always reached even at high multiplicities of R if the assay is left to completion (results not shown). Addition of 1000 ng of cytochrome *C*, which has a similar size and p*I* to the R domain, showed no inhibition of colicin N activity.

The in Vitro Affinity of R for OmpF Is Lower than That of Colicin N. Isothermal titration microcalorimetry (ITC) was used here to try and determine the dissociation constant and heat of binding for R and OmpF. Protein concentrations at which intact colicin N titrates (Evans *et al.*, 1996a) (400 μM colicin N and 15 μM trimeric OmpF, respectively) were too low to observe binding for either R, TR, or the proteolytically produced RP which is known to bind OmpF (El-Kouhen *et al.*, 1993, 1994) (data not shown). By increasing the

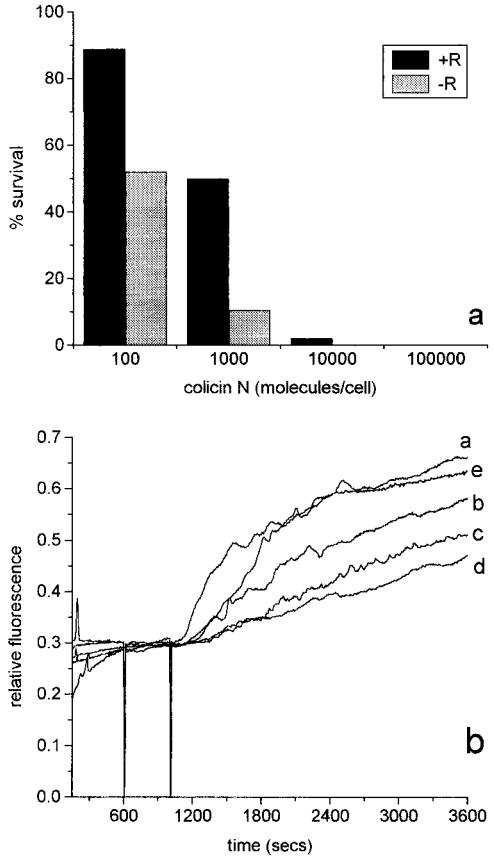

FIGURE 4: Protection of B^E3000 from colicin N by the R domain. (a) Cell survival assay. Sensitive cells were preincubated with either R at 25 μM or phosphate buffer prior to the addition of colicin N in 10-fold dilutions. Cell survival was evaluated from the $OD_{600\ nm}$ after 2 h following the addition of fresh LB. (b) ANS fluorescence assay. Spectra show the effect of the preincubation of cells with R domain on the fluorescence change of the probe ANS, which arises from the addition of 25 ng colicin N to sensitive B^E3000 cells: (a) no added R; (b) 50 ng of R; (c) 250 ng of R; (d) 1000 ng of R; (e) 1000 ng of cytochrome *c* (control).

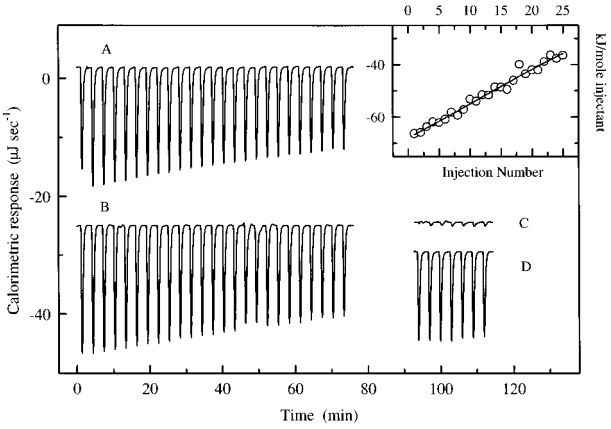

FIGURE 5: Isothermal calorimeter titration data for binding of ligands to OmpF at 25 °C. (A) Addition of ligand R (1.0 mM, 25 × 10 μL injections) to OmpF (62 μM trimer concentration, 1.4 mL). (B) Addition of ligand RP (0.7 mM, 25 × 10 μL injections) to OmpF (62 μM trimer concentration, 1.4 mL). (C) Ligand dilution control. (D) OmpF dilution control. Insert: Example of data fitting. Integrated heat effects for OmpF + RP, corrected for dilution controls and fit assuming identical binding sites. The solid line is the theoretical fit for $n = 3$, with binding constant (K_a) $\approx$ 10 000 M^{-1} ($K_d \approx 100$ μM) and enthalpy (ΔH) ≈ -105 kJ mol^{-1}.

concentrations of R and RP to 700−1000 μM and OmpF to 60 μM, binding for these domains could be observed (Figure 5), with relatively weak exothermic binding effects. The data are consistent with 3 R/RP binding per OmpF trimer; however, the effects are rather small and do not sufficiently approach binding saturation to yield reliable enthalpy (ΔH) estimates. Nevertheless, dissociation constants for both R and RP can be estimated from ITC to be approximately 100 μM, compared to 2 μM for whole colicin N (Evans *et al.*, 1996).

DISCUSSION

The results presented here demonstrate that the receptor domain (R) and the receptor plus translocation domains (TR) of colicin N can be expressed as isolated stable domains. Both have secondary structure in keeping with native colicin N, and retain an *in vivo* porin-binding site, binding to the OmpF and PhoE producing strains B^E3000 and CE1197. It thus seemed likely that R would be able to protect sensitive cells from colicin N attack by blocking all the receptor-binding sites. The cell survival assay shows that high concentrations of R do protect B^E3000 cells against colicin N attack. However, the protection compared to vitamin B_{12} (Cavard, 1994) and OmpF monoclonal antibodies (Bourdineaud *et al.*, 1990) was very low. At best, the cloned R domain could have the same affinity for OmpF as whole colicin N, but it is more likely that its affinity is actually lower, whereas antibody-binding affinities are usually on the order of 10^9 M^{-1}, i.e., much stronger than the affinity of colicin N for receptors, and therefore the limited protection afforded by R is unsurprising. In the ANS assay, R delays colicin activity but does not prevent it. Perhaps R, which is unable to translocate, does not stably block the porin-binding site but is in rapid exchange. Since colicins are "single hit killers", it only needs the replacement of one R with colicin N to occur to kill the cell.

Previously the only domain for which individual stability information was available was the pore-forming domain. For colicins A, E_1, and Ia, these domains have been shown to partially unfold in an acidic environment, losing tertiary but not secondary structure (Mel & Stroud, 1993; Muga *et al.*, 1993; Schendel & Cramer, 1994; van der Goot *et al.*, 1992). The unfolding of the pore-forming domain is probably a prerequisite for membrane insertion, and indeed low pH has been shown to increase the insertion rate for colicin Ia (Mel & Stroud, 1993), and for colicins A (Gonzalez-Manas *et al.*, 1992) and B (Evans *et al.*, 1996b). Recently we have shown that the pore domain of colicin N does not undergo this transition to an acid-unfolded state (Evans *et al.*, 1996b). Obviously the receptor domain of colicins has a very different function, and it was important to observe the effect of pH on the receptor domain of colicin N. No loss in secondary structure occurred, and although changes in the tertiary structure were apparent from the near-UV CD, there was no significant loss of tertiary structure. Hence, R appears to be a highly stable unit unaffected by acidic environments. This is probably an important feature in its role of receptor recognition at the negatively charged lipopolysaccharide membrane surface. Such stability does not however fit with our belief that colicins must unfold in order to translocate across the outer membrane. Examination of the structure indicates that the 63 Å helix which joins the R and P domains could unravel to connect an external (folded) R domain to a cytoplasmic membrane-inserted P domain. Data are required

which show which parts of the colicin N molecule cross the outer membrane.

The *in vitro* affinity of R for OmpF was surprisingly low (50-fold less than for colicin N), considering that it retains both its native structure and its porin recognition site. Since the proteolytically produced RP, which has been shown to bind OmpF *in vivo* (El-Kouhen *et al.*, 1993) and *in vitro* (El-Kouhen *et al.*, 1994), had a similarly low affinity, it seems unlikely that this is a result arising from the subcloning of the domain. In addition, while a low affinity could reflect an incomplete domain, extending R in either direction made little difference since neither TR nor RP bound tightly to OmpF. Instead, the micromolar affinity and high enthalpy observed for colicin N previously (Evans *et al.*, 1996) appear to be a product of the entire toxin molecule. As discussed earlier, the interaction of R with OmpF appears to be reversible, and perhaps in the whole toxin, the combination of T and P together plays a role in strengthening the interaction. Studies on colicin A suggest that the domains are not wholly independent since modifications on the pore-forming domain affect translocation (Duché *et al.*, 1994). In addition, there has been recent evidence suggesting that the T and P domains of colicin E_1 interact with each other (Salwinski *et al.*, 1993) in the native protein, and maybe this is also the case for colicin N. This would support the published low-resolution X-ray structure of colicin Ia which shows that the pore-forming domain is situated close to a probable N-terminal domain (Ghosh *et al.*, 1994). In addition, the voltage dependence and membrane fusion activities of colicin A and its pore-forming domain are different (Frenette *et al.*, 1989; Martinez *et al.*, 1983). On the other hand, whatever the role of domain interaction in receptor binding and translocation, it does not affect the insertion into model lipid vesicles. The insertion rates as measured by brominated phospholipid quenching of either the entire colicin A or its pore-forming domain are identical (Gonzalez-Manas *et al.*, 1992).

In summary, the receptor domain contains the recognition site for porins and is able to bind reversibly to OmpF. However, the binding affinity is greatly reduced compared to that of whole colicin N, and it is not increased by either the pore-forming domain or the translocation domain alone. This may indicate that *in vivo*, the function of the receptor domain of colicin N is not completely differentiated from that of the translocation and pore-forming domains.

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