Colicin Pore-Forming Domains Bind to *Escherichia Coli* Trimeric Porins†

Lynn G. Dover,‡ Lucy J. A. Evans, Susan L. Fridd, Graeme Bainbridge,§ Elaine M. Raggett,|| and Jeremy H. Lakey†,*

School of Biochemistry & Genetics, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, United Kingdom

Received January 24, 2000; Revised Manuscript Received May 3, 2000

ABSTRACT: Colicin N kills sensitive *Escherichia coli* cells by first binding to its trimeric receptor (OmpF) via its receptor binding domain. It then uses OmpF to translocate across the outer membrane and in the process it also needs domains II and III of the protein TolA. Recent studies have demonstrated sodium dodecyl sulfate- (SDS) dependent complex formation between trimeric porins and TolA-II. Here we demonstrate that colicin N forms similar complexes with the same trimeric porins and that this association is unexpectedly solely dependent upon the pore-forming domain (P-domain). No binding was seen with the monomeric porin OmpA. In mixtures of P-domain and TolA with OmpF porin, only binary and no ternary complexes were observed, suggesting that binding of these proteins to the porin is mutually exclusive. Pull-down assays in solution show that porin−P-domain complexes also form in the presence of outer membrane lipopolysaccharide. This indicates that an additional colicin−porin interaction may occur within the outer membrane, one that involves the colicin pore domain rather than the receptor-binding domain. This may help to explain the role of porins and TolA-II in the later stages of colicin translocation.

Colicins are protein bacteriocins produced by *Escherichia coli* to kill competing bacteria (*1*). The pore-forming colicins kill sensitive *E. coli* cells by creating ion channels in their inner membranes (*2*). The other major group of colicins kill by inserting a nuclease domain into the cytoplasm of the target cell, and in both groups the toxic activity is found in the C-terminal of three domains (*3*). The remaining two domains are required for outer membrane receptor binding (R domain) and translocation (**T**) across the outer membrane. There are eight known pore-forming colicins that show significant sequence and structural homology in the **P** domain but poor homology beyond this region (*4*). The **T** and **R** domains thus appear to be the result of recruitment and divergent evolution, and this is understandable since the pore-forming colicins enter cells via many different surface receptors and at least two different translocation routes; the Tol- (*5*) (group A colicins; *6*) and Ton-dependent pathways (*7*) (group B; *8*). Colicin N, used here, binds to TolA domain III (TolA-III) via its T-domain (*9*) and also needs TolA-II for full activity (*10*).

The mechanism by which these large proteins (42−70 kDa) can traverse the outer membrane is not understood. However for colicins A and N, is it clear that the outer membrane porin protein OmpF acts as both a cell surface receptor that binds the **R**-domain *and* a translocation pathway for the **P**-domain (*11*−*13*). It is also clear that this pathway is not through the pore lumen (*14*). Interestingly, there is very good evidence that colicin A spans the *E. coli* periplasm at contact sites enriched in TolA (*15*).

There have been reports in recent years that two Tol proteins, A (*16*) and B (*17*), from the colicin translocation pathway bind to porins in the presence of SDS. This interaction has been proposed as important in the OmpF maturation pathway, being reminiscent of the SDS-induced binding of porin to the periplasmic chaperone Skp (*18*). In this paper we show that pore-forming domains of colicins bind porins under conditions identical to those published for TolA and B (*16*, *17*). The Tol proteins also appear to bind at the same site as the colicins. Furthermore, we show that this interaction can be measured in solution where SDS can be replaced by a natural outer membrane component, lipopolysaccharide.

MATERIALS AND METHODS

Protein Purification. Colicin N and was purified from *E. coli* BZB1019 (hsdR) carrying pChap4 as previously described (*19*). The production of the 6-His-tagged *R* and *TR* of colicin N in *E. coli* JM103 by use of a modified pET8c vector has been previously described (*19*). The α-chymotryptic (*RP*) and thermolytic (P_N) fragments of colicin N were purified as described previously (*19*). His-tagged TolR-II was prepared as published from a plasmid supplied by Lutz Riechmann (*20*). OmpF and PhoE were purified as previously described from *E. coli* BE 3000 and *E. coli* CE1197, respectively (*13*). OmpA, LamB, and P_B were gifts from F.

† We thank the Wellcome Trust (L.G.D. and L.J.A.E.) and BBSRC (S.L.F. and G.B.) for financial support. E.M.R. was funded by a University of Newcastle, Faculty of Medicine Luccock Research Studentship.

* Corresponding author: e-mail: J.H.Lakey@ncl.ac.uk; tel (0191) 222-8865; fax (0191) 222-7424.

‡ Present addresses: Department of Microbiological, Immunological and Virological Sciences, University of Newcastle upon Tyne.

§ Present address: Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ, U.K.

|| Present address: Dairy Products Research Centre, Teagasc, Moorepark, Fermoy, Cork, Ireland.

10.1021/bi000160n CCC: $19.00
Published on Web 06/24/2000

Pattus, and TolA-II−III (*5*) was prepared as previously described. Colicin E_9 (*3*) was a gift from K. Kleanthous and R. James. All protein concentrations were determined by UV absorption at 280 nm. Molecular mass (in kilodaltons) and molar extinction coefficients (ϵ;ion liters per mole per centimeter) are as follows: colicin N (42, 49 500), P_N (22, 23 470), P_B (22, 23 470), T (10, 12 660), R (15; 13 370), TR (22; 26 030), RP (35; 36 840), LamB (47; 136 390), OmpF (37, 48 500), OmpC (38, 59 880), PhoE (37; 45 230), OmpA (35, 50 330), TolA-II−III (38, 6520), TolB (44, 56 470), and colicin E_9 with its immunity protein (62, 47 510 + 10, 9590).

Antibodies. The polyclonal antiserum used to detect OmpF has been described previously (*14*). The polyclonal antiserum used to detect P_N was raised in rabbits against denatured whole colicin N. The polyclonal antiserum used to detect TolA-II−III was raised in rabbits against TolA-III and was a kind gift from Roland Lloubès (*16*).

UV Circular Dichroism Spectra of P_N. The near UV−CD spectra of P_N at 13 μM in 0.0625 M Tris-HCl and 0.3 M NaCl, pH 6.8, with and without 1% SDS, were recorded between 250 and 320 nm at 20 °C on a Jobin Yvon CD6 spectrometer with a 1 cm path length cuvette. Baseline spectra for the buffer with and without SDS were subtracted from the appropriate experimental spectra. For measuring far UV−CD spectra (194−250 nm) a 0.2 mm path length cuvette was used.

Complex Formation in the Presence of SDS. The proteins were mixed in SDS−PAGE sample buffer (containing 2% SDS, giving a final SDS concentration of 1% and without β-mercaptoethanol) and adjusted to a final NaCl concentration of 0.5 M to prevent porin aggregation. Incubations were initially carried out for 1 h at 37 °C but were found to be complete after 2 min at ambient temperature. Unless stated otherwise, samples were not boiled and were run on SDS−12% polyacrylamide gels immediately. Protein bands were visualized with Coomassie Brilliant Blue R-250. For Western analysis proteins were transferred to nitrocellulose (0.45 mm pore size) by standard semidry techniques. The blots were incubated with a suitable dilution of the relevant antiserum. Bands were visualized by use of an anti-rabbit IgG (whole molecule)−horseradish peroxidase conjugate (Sigma).

Pull-down Assays. Pull-down experiments used the methods of ref *16*. All samples contained PN buffer [100 μL of 100 mM NaCl and 50 mM Tris, pH 8.0, supplemented with 1% (w/v) SDS, octyl-POE, or LPS], 5 μL of suspended NTA−agarose beads (Qiagen), and 10 μg of OmpF. 6-His-tagged colicin N P-domain or TolR−II ("Bead") (5 μg) and 10 μg of TolAII−III or colicin A ("Soln") were added where indicated. Samples were incubated in 500 μL reaction tubes for 45 min at room temperature before being washed three times in PN buffer. (PN wash buffer for the LPS experiment contained 0.25% LPS) Finally, the proteins attached to the pelleted beads were boiled in SDS−PAGE sample buffer, separated by SDS−PAGE, and stained with Coomassie blue. Octyl-POE is Octyl poly(oxyethylene) (Bachem, Switzerland) and LPS is lipopolysaccharide from *E. coli* O55:B5 (Sigma, Poole, U.K. L2880).

RESULTS

Pore-Forming Colicins Form Stable Complexes with Trimeric Porins in SDS. The methods used were essentially as previously published (*16*). When the porins and colicin N fragments were mixed together in the presence of 1% SDS and separated on SDS−PAGE, domain-specific interactions were observed (Figure 1A). The migration of OmpF and PhoE through the gel was retarded upon mixing with colicin N, RP, or P_N in SDS, suggesting the formation of a complex between *P* and both porins. With the *TR* domain a very faint higher molecular weight band was seen with most of the porin remaining free, indicating the formation of a weakly associated complex. The *R* domain, however, showed no complex formation at all.

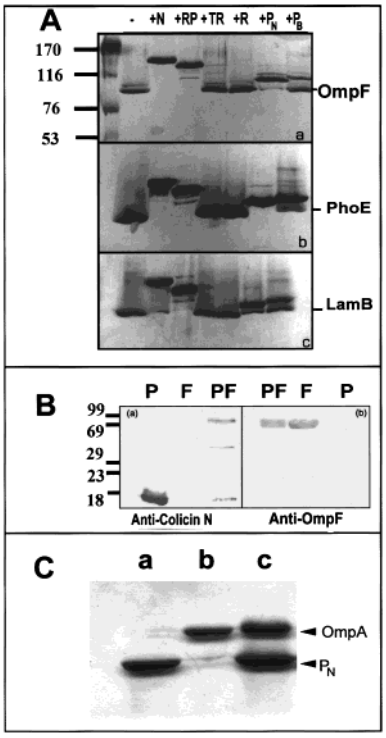

FIGURE 1: Complexes formed by domains of colicins N and B with various porins. (A) Various colicin domains were incubated with porins in sample buffer for 1 h. Unboiled samples were analyzed by SDS−PAGE. Complex formation is indicated by the retarded bands in lanes +N (M_r = 42 kDa), +RP (M_r = 35 kDa), and +P (M_r = 22 kDa). (a) OmpF complexes; (b) PhoE complexes; (c) LamB complexes. Lane 1, molecular size standards; lane 2, free porin trimer; lane 3, porin + colicin N; lane 4, porin + *RP*; lane 5, porin + *TR*; lane 6, porin + *R*; lane 7, porin + P_N; lane 8, porin + P_B. OmpC formed complexes as OmpF in panel a. To resolve the complexes that vary by only 10−30 kDa over 120 kDa, the PAGE gels require extended separation times, which cause the smearing of the unbound porin trimer bands. The smearing is reduced in the complexes, suggesting more compact structures. (B) $^{+Ib}$LPS−OmpF (40 pmol) was incubated with 240 pmol P_N for 2 min at ambient temperature before separation by SDS−PAGE and immunoblotting. Panel a was probed with a rabbit polyclonal serum raised against whole denatured colicin N; panel b was probed with a rabbit polyclonal serum raised against an OmpF peptide. P denotes P_N only; F, OmpF only; and PF, OmpF + P_N. Colicin N antiserum revealed a species with intermediate mobility between the complex and free P_N that was not detected unless the two proteins were mixed. This is possibly a minor fraction of a monomeric OmpF capable of binding P_N that was not detected with the OmpF antiserum as a consequence of its being raised against a peptide, thereby limiting the number of potential epitopes per mole of antigen compared to that of the colicin N serum. (C) P_N (400 pmol) was mixed with 40 pmol of OmpA and held at ambient temperature for 2 min before analysis by SDS−PAGE on 12% gels. Lane a, P_N only; lane b, OmpA; and lane c, OmpA + P_N. OmpA is only slightly retarded.

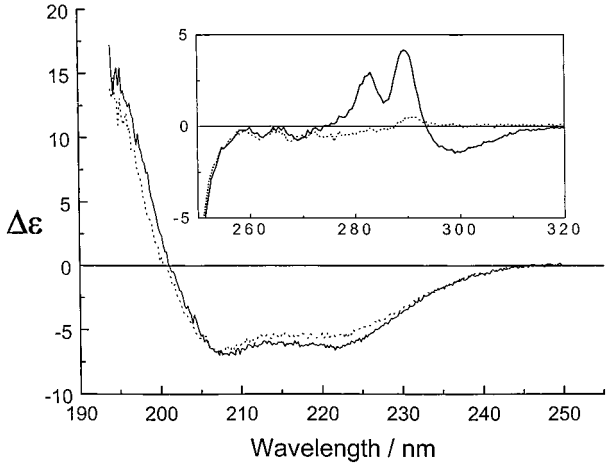

FIGURE 2: Circular dichroism analysis of P_N. The near (inset; 250−320 nm) and far (194−250 nm) UV−CD spectra of P_N in 0.0625 M Tris-HCl and 0.5 M NaCl, pH 6.8, with (dashed line) and without (solid line) 1% SDS were recorded at 20 °C in 1 cm (near) and 0.2 mm (far) path length cuvettes, respectively, at a protein concentration of 13 μM.

We also incubated the P-domain of colicin B (P_B) with PhoE and OmpF (Figure 1Aa,b). P_B has >50% sequence identity to P_N (*21*), but colicin B uses FepA [Fe(III)-enterobactin receptor] as its receptor and is Ton-dependent (*22*). P_B also formed complexes with both porins, although free porin can also be seen, possibly indicating a weaker interaction than occurs with P_N. P-dependent complexes formed with maltoporin, an 18-stranded β-barrel trimer (*23*), suggesting that this colicin−porin interaction takes place between any P and any trimeric porin. This behavior is identical to the observed TolA-II−OmpF binding (*16*). Western blots of OmpF and P_N complexes (Figure 1B) confirmed the presence of P_N in a high molecular weight complex with OmpF.

Pore-Forming Domain Does Not Bind to the Monomeric Porin OmpA. When mixed with P_N in 1% SDS, OmpA showed slightly reduced migration (Figure 1C) but analysis by Western blotting did not reveal comigration of P_N with OmpA (not shown). Thus only trimeric porins form stable complexes with P_N under these conditions. The studies thus far were all carried out with $^{-lb}$LPS-porins (i.e., without loosely bound LPS). In all of the following investigations the use of $^{+lb}$LPS-porins demonstrates that these complexes can be formed with both porin isoforms.

Detergent-Induced Tertiary Structure Alteration of P_N. The near UV−CD spectrum of native P_N has strong positive peaks indicative of aromatic residues constrained by a rigid tertiary structure (Figure 2, inset) (*24*). In 1% SDS these peaks were lost and replaced with a broad, featureless, and negative signal indicating a loss of tertiary structure (*25*). Far UV−CD spectra (Figure 2) showed comparatively little change in the spectrum of this predominantly α-helical domain on the introduction of the detergent. The changes are similar to those observed upon molten globule formation and membrane insertion in colicin A (*25*). Similar data were obtained in 1% octyl-POE (data not shown).

TolA-II−III Can Displace P_N from Its Complex. The colicin translocator TolA consists of three domains; the first-TolA-I, is a cytoplasmic membrane-spanning domain and hence experiments were carried out with TolA-II−III (*5*).

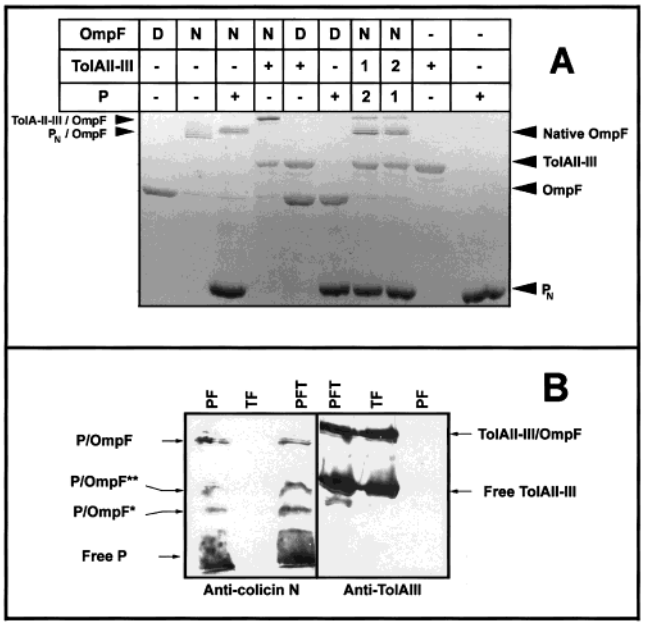

FIGURE 3: Interactions of P_N and TolA-II−III with native and denatured $^{+lb}$LPS−OmpF. (A) In each case loadings for the various ligands were (a) OmpF, 0.6 nmol; (b) TolA-II−III, 6 nmol; and (c) P_N, 6 nmol. OmpF was denatured by heating at 95 °C for 5 min, where necessary, before mixing with ligands. All incubations were for 2 min at ambient temperature. Key: D denotes denatured OmpF; N, native OmpF; +, ligand addition; −, absence of ligand; 1, first addition; 2, second addition. (B) OmpF trimers (10 pmol) were mixed with P_N at a molar ratio of 10:1 (P_N:OmpF) in 1% SDS and 0.5 M NaCl at ambient temperature. After 2 min, TolA-II−III was also added the molar ratio of 10:1. Single ligand mixes with OmpF were also run to discount immunochemical cross-reactions. The left-hand panel was probed with anti-colicin N and the right with anti-TolA-III. PF denotes OmpF + P_N; TF, OmpF + TolA-II−III; PFT, OmpF + P_N + TolA-II−III. The lower band only seen with anti-TolA-III in PFT is presumably the breakdown product of TolA-II−III, which is visible in Coomassie Blue-stained gels (A). In TF it migrates with OmpF but is displaced in PFT. P/OmpF** and P/OmpF* denote bands that may correspond to the complexes of P_N with minor fractions of monomeric and dimeric porin.

TolA-II−III and P_N formed complexes with native OmpF in SDS that were easily distinguishable by electrophoresis, since TolA-II−III causes significantly greater retardation than P_N (*16*). Porin denatured by boiling in SDS for 5 min did not form a complex (Figure 3A). TolA-II−III and P_N showed no mobility shifts when incubated together in SDS in the absence of OmpF (not shown).When either TolAII−III or P_N was incubated with OmpF alone followed 2 min later by the other protein, two separate complexes were detected on SDS−PAGE that migrated identically to the TolA-II−III/OmpF and P_N/OmpF complexes, respectively (Figure 3A).

For each complex, conditions were found that resulted in complete retardation of the OmpF, showing saturation of the binding. To confirm that the two distinct complexes contained a single ligand species, we prepared samples in which the P_N/OmpF complex was formed, followed by addition of an equimolar (cf. P_N) amount of TolA-II−III. After SDS−PAGE, the resulting Western blot was split and the identical parts were probed with either TolA-III or colicin N-specific antisera (Figure 3B). The blot demonstrated that each antiserum reacted with only one of the two distinct high molecular weight bands, confirming that these were indeed distinct TolA-II−III/OmpF and P_N/OmpF complexes. This

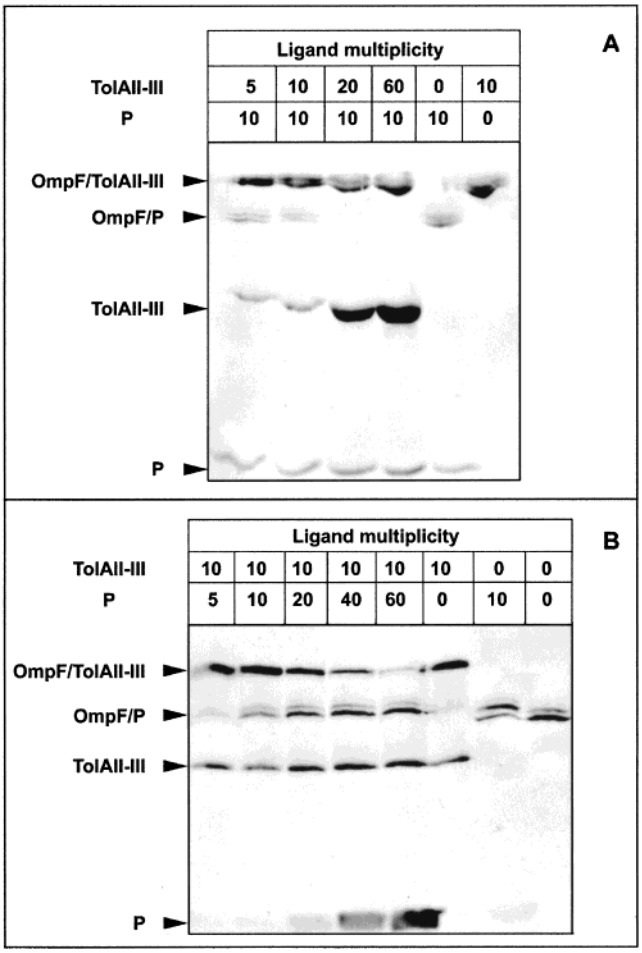

FIGURE 4: Concentration-dependent porin ligand exchange. (A) Exclusion of P_N from its porin complex by increasing concentrations of TolA-II−III. OmpF trimers (110 pmol) were mixed with P_N at a molar ratio of 10:1 (P_N:OmpF) in 1% SDS and 0.5M NaCl at ambient temperature. After 2 min, TolA-II−III was added to the molar ratios shown above each lane. (B) Exclusion of TolA-II−III from its porin complex by increasing concentrations of P_N. OmpF trimers were (110 pmol) mixed with TolA-II−III at a molar ratio of 10:1 (TolA-II−III:OmpF) in 1% SDS and 0.5 M NaCl at ambient temperature. After 2 min, P_N was added to the molar ratios shown above each lane. In both cases the reaction mixes were loaded onto SDS−PAGE 12% gels and electrophoresed immediately.

is clear evidence against the formation of a ternary complex. Bands were seen on the blot that may correspond to the P_N bound to monomers and dimers of OmpF. These bands were never visible on Coomassie blue stained SDS−PAGE. Following complex formation between OmpF and either TolA-II−III and P_N under saturating conditions, increasing concentrations of the other ligand were introduced (Figure 4). In each case, both complexes formed and as the concentration of the second ligand increased, its complex with OmpF became increasingly prominent. These observations are consistent with a dynamic system in which TolA-II−III and P_N bind OmpF in an exchangeable manner. TolA-II−III appeared to be slightly more efficient in displacing P_N than vice versa.

Titration of the OmpF/TolB complex reported by ref *17* was reproduced in 1% SDS and the addition of an equimolar (cf. TolB) amount of P_N resulted in complete conversion of the porin to an OmpF/P_N complex (not shown). When equimolar TolB (cf. P_N) was added to OmpF/P_N, no displacement of the colicin domain from its porin complex

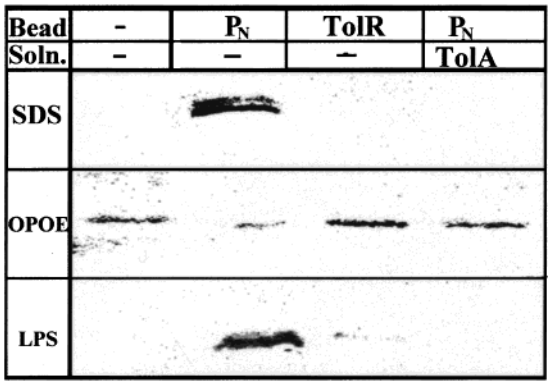

FIGURE 5: SDS and LPS cause porin−colicin N complexes in pull-down assays. Pull-down experiments used the methods of ref *16*. All samples contained PN buffer [supplemented with 1% (w/v) SDS, octyl-POE, or LPS], 5 μL of suspended NTA−agarose beads (Qiagen), and 10 μg of OmpF. 6His-tagged P_N or TolRII (5 μg) ("Bead") and 10 μg of TolAII−III ("Soln") were added where indicated (see text). The bands on the gels show whether OmpF bound to the beads in each sample. Nonspecific binding was observed in octyl-POE and to a lesser extent in LPS. OmpF clearly binds to P_N in SDS and LPS, and this interaction is prevented by soluble TolA-II−III. The reason for the doublet band in the SDS samples is not known.

was detected. Therefore binding of P_N by OmpF is unaffected by the presence of TolB and again the behavior of P_N closely resembles that of TolA-II (*17*).

Colicin E_9 C-Terminal Domain Does Not Form an SDS-Inducible Complex with OmpF. To test whether SDS-inducible binding of trimeric porins is unique to pore-forming colicins we analyzed the OmpF-binding of a group A nuclease colicin (E_9) C-terminal domain (*3*) in 1% SDS (data not shown). There was no shift in the migration of either protein and therefore no evidence that this unrelated but largely helical domain formed any complex with OmpF. Thus the interaction with porins is clearly a property of the pore-forming domains rather than all colicin toxic domains.

Lipopolysaccharide Causes Colicin−Porin Complex Formation. To test the colicin−porin interaction in solution we used His(*6*)-tagged P_N (His-P_N) attached to NTA−agarose beads to "pull down" OmpF (Figure 5) (*17*). This occurred both when SDS was used and also when 1% LPS replaced the SDS. This reveals that complex formation can be promoted by a natural outer membrane component and is not peculiar to SDS treatment. To test the specificity of the interaction we used NTA−agarose without His-P_N, with His-TolRII (*20*), and with soluble TolA-II−III and His-P_N. In each case no binding was observed, showing that the interaction is identical to that observed on SDS−PAGE including the inhibition by TolA. As reported previously (*16*), only nonspecific binding was observed in octyl-POE. Characterization of the SDS-induced complex by gel-filtration chromatography was not possible as the P-domain in SDS migrates at $M_r = 60\,000$, which does not allow sufficient separation from the OmpF trimer. SDS-induced oligomerization has been observed in hydrodynamic studies of colicin A (*26*) but it is not seen in SDS−PAGE (our unpublished data).

DISCUSSION

The data presented here provide significant additional information about the SDS-dependent porin-containing com-

plexes that have been reported in recent years. The first example was that of the periplasmic chaperone Skp, which was reported to bind porins in a column affinity assay in SDS (*18*). Second, the periplasmic domain of TolA (TolA-II) was shown to bind all trimeric porins in the presence of SDS (*16*). Finally, it was shown that TolB shows similar behavior although with a lower affinity (*17*). Here we show that the P domains and the Tol proteins show clear competition in their binding to porins. This may mean either that they compete for an overlapping binding site or that long-range allosteric effects prevent simultaneous binding. The P-domain is shown to bind to several trimeric porins whereas the remaining domains of colicin N, and those of colicin E9, do not bind under these conditions. We also show that the outer membrane component LPS, which is necessary for porin maturation (*27*), can substitute for SDS, thus increasing the physiological relevance of the observed interactions. It is of relevance here that the Tol system's only clear function is the translocation of colicins across the outer membrane and periplasm, a process in which porins play a major role as well.

Our data and previous experiments have shown that in neutral detergent the pore-forming domains of colicins do not bind porins (*13*, *19*). Hence, anionic surfactants such as LPS and SDS are essential for the formation of the tight complex and currently it is impossible to determine whether this is due to the observed alteration of the tertiary structure of P_N or an as-yet unseen change in the porin trimer.

Neither porins nor Tol proteins have been implicated in colicin B activity so it was surprising that complexes were seen when the pore-forming domain of colicin B (P_B) was used in place of P_N. The pore-forming domains of colicins N (*28*), A (*21*), E_1 (*29*), and Ia (*30*) have similar folds, so P_B is likely to be very similar. Thus one common element of P-domains is the buried very hydrophobic helix pair surrounded by eight amphipathic helices. The 3D structures of OmpF, PhoE (*31*), and maltoporin (*23*) are known and the binding of P_N to the latter was most unexpected since it shows no homology to OmpF-type porins. All, however, are trimeric amphipathic β-barrels exposing only hydrophobic residues to the membrane phase.

Under saturating conditions, complexes of OmpF with P_N and with TolA-II−III were easily distinguishable on SDS−PAGE. The displacement of the porin-bound P_N from its complex by TolA-II−III and vice versa suggests a direct competition between P_N and TolA-II−III for OmpF binding, which allows us to be more confident of a functional significance for these data. Together with the observation of TolB displacement from porins by TolA (*17*), the data indicate a specific interaction by three types of protein for a single or interacting sites on *E. coli* porins. Interestingly, E1, the only group A colicin that does not require OmpF for translocation, is also the only one not to require a complete TolA-II domain for its activity (*10*).

R_N−OmpF binding concentrates the colicin at the bacterial surface (*11*−*13*) via an aqueous phase protein−protein interaction that is inhibited by detergents (*19*, *32*). Hence the role of detergent/LPS-induced porin binding by P_N is not clear. Our best guess is that we may be viewing a trapped intermediate in the translocation pathway. In this state the colicin domain is interacting with the porin as it would during passage through the outer membrane. This agrees with previous data that peripheral regions of the OmpF trimer barrel are crucial for translocation (*33*) and that colicin N does not translocate through the center of the OmpF pore (*14*). If the complexes seen here are translocation intermediates, their displacement by TolA-II may offer a clue as to why this domain is important for toxicity of most group A colicins. The repetitive sequence of the TolA-II sequence which is responsible for the porin binding consists of 10 tandem repeats of [ED]-$K_{(1,2)}$-$A_{(2,4)}$ with a coiled-coil propensity, e.g., (217-EKAKAEAEKKAAAEKAAA-234). In the pore-forming regions of the colicins tested, the closest match is in the N-terminal region, which links to the receptor-binding domain (colicin N 184-RKEEKEKNEKEALLKA-199). This section of the P-domain is not required for pore formation (*34*) and in several published models of pore-forming colicin translocation it is shown adjacent to the porin (*28*, *35*).

SDS−PAGE Artifact or Useful Biological Data? Porins are fully active in SDS and the data here and elsewhere (*36*) indicate that, under the same conditions, colicins are converted into the membrane insertion-competent state. Hence the SDS experiments are not carried out under denaturing conditions for either protein. However, boiling the samples does prevent the interactions.

The pull-down assays show that the polyacrylamide gel is not required to form the complex and that SDS can be replaced by a physiologically relevant, negatively charged amphiphile, LPS. This solution-state binding is also inhibited by TolA-II−III in the same way as the SDS−PAGE effect. *In vivo*, the interaction of the P-domain with porins will take place in the presence of LPS so this experiment comes close to reproducing the natural state. Hence, from this and the previously published data (*16*, *17*) we believe that the binding occurs under conditions that make it a serious candidate for one of the porin−colicin interactions necessary for translocation.

Comparison of P-Domains with the Bcl Family. The link between these two families has been evident since the Bcl-x_L structure was solved and the helical bundle with a buried hydrophobic helical hairpin, first seen in colicin A, was found in the Bcl-2 family (*37*). The proteins also share several characteristics such as pore formation. It was recently shown that "pore domain like" Bcl and Bax proteins show competitive binding to the mitochondrial "porin" VDAC (*38*). It is therefore interesting that this protein motif might to show a general affinity for β-barrel membrane proteins.

ACKNOWLEDGMENT

The excellent technical work of Pauline Heslop is gratefully acknowledged. We thank F. Pattus and V. Braun for OmpA and colicin B samples, C. Kleanthous and R. James for colicin E_9 samples, R. E. Webster for TolA-producing strains, L. Riechmann for TolR-II, and R. Lloubès for supplying Anti-TolA-III antibodies. The advice and encouragement of Bob Lightowlers is much appreciated.

REFERENCES

1. Lakey, J. H., van der Goot, F. G., and Pattus, F. (1994) *Toxicology 87*, 85−108.
2. Schein, S. J., Kagan, B. L., and Finkelstein, A. (1978) *Nature 276*, 159−63.

3. Kleanthous, C., Kuhlmann, U. C., Pommer, A. J., Ferguson, N., Radford, S. E., Moore, G. R., James, R., and Hemmings, A. M. (1999) *Nat. Struct. Biol. 6*, 243−252.
4. Riley, M. A. (1998) *Annu. Rev. Genet. 32*, 255−278.
5. Webster, R. E. (1991) *Mol. Microbiol. 5*, 1005−11.
6. Davies, J. K., and Reeves, P. (1975) *J. Bacteriol. 123*, 102−17.
7. Pilsl, H., and Braun, V. (1995) *Mol. Microbiol. 16*, 57−67.
8. Davies, J. K., and Reeves, P. (1975) *J. Bacteriol. 123*, 96−101.
9. Raggett, E. M., Bainbridge, G., Evans, L. J. A., Cooper, A., and Lakey, J. H. (1998) *Mol. Microbiol. 28*, 1335−1344.
10. Schendel, S. L., Click, E. M., Webster, R. E., and Cramer, W. A. (1997) *J. Bacteriol. 179*, 3683−3690.
11. El-Kouhen, R., Fierobe, H. P., Scianimanico, S., Steiert, M., Pattus, F., and Pages, J. M. (1993) *Eur. J. Biochem. 214*, 635−639.
12. Fourel, D., Hikita, C., Bolla, J. M., Mizushima, S., and Pages, J. M. (1990) *J. Bacteriol. 172*, 3675−80.
13. Evans, L. J. A., Cooper, A., and Lakey, J. H. (1996) *J. Mol. Biol. 255*, 559−563.
14. Bainbridge, G., Armstrong, G. A., Dover, L. G., Whelan, K. F., and Lakey, J. H. (1998) *FEBS Lett. 432*, 117−122.
15. Guihard, G., Boulanger, P., Benedetti, H., Lloubès, R., Besnard, M., and Letellier, L. (1994) *J. Biol. Chem. 269*, 5874−5880.
16. Derouiche, R., Gavioli, M., Benedetti, H., Prilipov, A., Lazdunski, C., and Lloubès, R. (1996) *EMBO J. 15*, 6408−6415.
17. Rigal, A., Bouveret, E., Lloubès, R., Lazdunski, C., and Benedetti, H. (1997) *J. Bacteriol. 179*, 7274−7279.
18. Chen, R., and Henning, U. (1996) *Mol. Microbiol. 19*, 1287−1294.
19. Evans, L. J. A., Labeit, S., Cooper, A., Bond, L. H., and Lakey, J. H. (1996) *Biochemistry 35*, 15143−15148.
20. Riechmann, L., and Holliger, P. (1997) *Cell 90*, 351−360.
21. Parker, M. W., Postma, J. P., Pattus, F., Tucker, A. D., and Tsernoglou, D. (1992) *J. Mol. Biol. 224*, 639−57.
22. Hancock, R. E., Hantke, K., and Braun, V. (1976) *J. Bacteriol. 127*, 1370−5.
23. Schirmer, T., Keller, T. A., Wang, Y. F., and Rosenbusch, J. P. (1995) *Science 267*, 512−514.
24. Evans, L. J. A., Goble, M. L., Hales, K., and Lakey, J. H. (1996) *Biochemistry 35*, 13180−13185.
25. van der Goot, F. G., González-Mañas, J. M., Lakey, J. H., and Pattus, F. (1991) *Nature 354*, 408−10.
26. Cavard, D., Sauve, P., Heitz, F., Pattus, F., Martinez, C., Dijkman, R., and Lazdunski, C. (1988) *Eur. J. Biochem. 172*, 507−12.
27. Bolla, J. M., Bernadac, A., Lazdunski, C., and Pages, J. M. (1990) *Biochimie 72*, 385−95.
28. Vetter, I. R., Parker, M. W., Tucker, A. D., Lakey, J. H., Pattus, F., and Tsernoglou, D. (1998) *Structure 6*, 863−874.
29. Elkins, P., Bunker, A., Cramer, W. A., and Stauffacher, C. V. (1997) *Structure 5*, 443−458.
30. Wiener, M., Freymann, D., Ghosh, P., and Stroud, R. M. (1997) *Nature 385*, 461−464.
31. Cowan, S. W., Schirmer, T., Rummel, G., Steiert, M., Ghosh, R., Paupit, R. A., Jansonius, J. N., and Rosenbush, J. P. (1992) *Nature 358*, 727−733.
32. Stora, T., Lakey, J. H., and Vogel, H. (1999) *Angew. Chem., Int. Ed. Engl. 38*, 389−392.
33. Fourel, D., Mizushima, S., Bernadac, A., and Pages, J. M. (1993) *J. Bacteriol. 175*, 2754−2757.
34. Baty, D., Lakey, J., Pattus, F., and Lazdunski, C. (1990) *Eur. J. Biochem. 189*, 409−13.
35. Cramer, W. A., Heymann, J. B., Schendel, S. L., Deriy, B. N., Cohen, F. S., Elkins, P. A., and Stauffacher, C. V. (1995) *Annu. Rev. Biophys. Biomol. Struct. 24*, 611−641.
36. Merrill, A. R., Cohen, F. S., and Cramer, W. A. (1990) *Biochemistry 29*, 5829−36.
37. Muchmore, S. W., Sattler, M., Liang, H., Meadows, R. P., Harlan, J. E., Yoon, H. S., Nettesheim, D., Chang, B. S., Thompson, C. B., Wong, S. L., Ng, S. C., and Fesik, S. W. (1996) *Nature 381*, 335−341.
38. Shimizu, S., Narita, M., and Tsujimoto, Y. (1999) *Nature 399*, 483−487.

BI000160N