Isolation and Preliminary Characterization of Wild-Type OmpC Porin Dimers from *Escherichia coli* K-12

Warren J. Rocque and Estelle J. McGroarty*

Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824

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ABSTRACT: *Escherichia coli* K-12 strain PLB3255 contains a mutation in the *ompF* gene that results in a 15 amino acid deletion in the porin protein. The mutation (*dex*) appears to increase the OmpF channel size, allowing the PLB3255 cells to grow on maltodextrins in the absence of a functional maltoporin. Porin isolated from strain PLB3255, which contains a wild-type *ompC* gene, was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and shown to contain 50–60% trimer aggregates and 35–40% of a 50-kDa "dimer". When the porin isolate was heated to 100 °C and separated on SDS gels containing 8 M urea, both the trimer and the "dimer" were recovered in a single band at 36 kDa that corresponded in mobility to wild-type OmpC porin. An analysis of the temperature stability of the isolate showed that the OmpC "dimer" was less stable and denatured at 66 °C compared to 81 °C for the trimer. To separate these aggregates, the unheated porin was suspended in 30% SDS, applied to a Sephadex G-200 gel filtration column, and eluted with 0.5% sodium deoxycholate. Two peaks were recovered containing separated trimers and "dimers". Circular dichroism spectra of isolated dimers and trimers indicated similar amounts of β-structure. The isolated dimers and trimers were reconstituted into artificial membranes. Electrical conductance across planar bilayer lipid membranes and liposome swelling assays showed that the two isolates had similar channel-forming activity. Four other *ompF* deletion mutants of the same phenotype were also shown to produce 50-kDa OmpC porin "dimers". In contrast, an *ompC* deletion mutant and an *ompF* point mutant, each of which produced a porin with an expanded channel, showed no evidence of "dimer" formation, indicating that dimers are not involved in forming the expanded channel. These studies indicate that specific changes in the structure of OmpF porin can alter the assembly of the OmpC protein. Furthermore, we found that the "dimer" is a stable porin configuration, and this "dimer" may represent the smallest functional, channel-forming aggregate of the OmpC protein.

The outer membrane of *Escherichia coli* K-12 contains two major channel-forming proteins, the OmpC and OmpF porins. These porins form water-filled diffusion channels with a size-exclusion limit of 600 Da. Electron microscopy, chemical cross-linking, and sedimentation equilibrium studies have shown that porins exist in the outer membrane as trimers of 36-kDa polypeptide subunits. Both porins are tightly bound to the peptidoglycan (Rosenbusch, 1974; Hasegawa et al., 1976) and to lipopolysaccharide (LPS;[1] Yamada & Mizushima, 1980; Rocque et al., 1987), and they are unusually stable to a wide variety of perturbants including high temperature (Rosenbusch, 1974), SDS (Nakae et al., 1979; Yu et al., 1979), and urea (Yu et al., 1979).

Previous studies have indicated that the trimer is the smallest functional channel-forming unit for all *E. coli* (Nakae et al., 1979) and *Salmonella typhimurium* porins (Tokunaga et al., 1979; Ishii & Nakae, 1980). Monomers obtained by denaturing the isolates show no channel-forming activity (Nakae et al., 1979). However, monomers and dimers within trimeric complexes appear to be functional in planar bilayer lipid membrane studies when the protein subunit interactions are weakened by low pH and high voltage (Xu et al., 1986). Although many of the physical properties of porins are well characterized, very little is known about the assembly and export mechanisms involved in targeting these proteins to the outer membrane. The kinetics of OmpF porin synthesis, processing, and assembly have recently been described by Reid et al. (1988). This study reported that a 50-kDa dimer was an intermediate in the assembly of OmpF trimers. Other studies have shown that overexpression of the OmpC porin results in an almost complete inhibition of transport of OmpA and LamB proteins to the outer membrane (Click et al., 1988). Furthermore, a mutation within the *ompC* gene has been shown to affect the expression of many outer membrane proteins (Catron & Schnaitman, 1987). The transport of these mutant or overexpressed porins to the outer membrane appears to jam the export machinery, preventing the export of other outer membrane proteins.

Analysis of specific mutations which alter porin structure can give information of the assembly and transport of these proteins as well as define the regions of the protein critical for function. Recently, several *E. coli* K-12 *ompF* deletion and point mutations have been characterized which allow for cells to grow on maltodextrins in the absence of the maltoporin, which is normally required for uptake of these larger polymers (Benson et al., 1988). Since the OmpC and OmpF wild-type porin channels cannot accommodate these larger maltodextrins, the *dex* mutations in the *ompF* gene result in an increase in the OmpF pore size. In this study, we have analyzed the structure and function of porins isolated from these *ompF dex* mutant strains. We show that *E. coli ompF dex* deletion mutants containing a wild-type *ompC* gene produce

* Correspondence should be addressed to this author.

[1] Abbreviations: LPS, lipopolysaccharide; Tris, tris(hydroxymethyl)aminomethane; SDS, sodium dodecyl sulfate; NaDOC, sodium deoxycholate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; BLM, bilayer lipid membrane.

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stable, functional wild-type OmpC "dimers".

Materials and Methods

Cell Growth and Porin Isolation. The strains used in this study were PLB3252, PLB3255, PLB3256, OC200, OC208, OC209, and ECB5084, all derivatives of *E. coli* MC4100, and were the generous gift of S. Benson. The first three strains have been described previously (Benson & Decloux, 1985). Strains OC200, OC208, OC209, and ECB5084 are derivatives of strain MCR106 lacking the *lamB* gene but containing a wild-type *ompC* gene and the mutant *ompF* gene of strains OC1500, OC1508, OC1509, and OC5104, respectively (Benson et al., 1988). Cells were grown in nutrient broth (1% tryptone, 0.2% yeast extract, and 0.4% NaCl) at 37 °C and harvested in late logarithmic phase. Outer membrane porins were isolated by the method of Lakey et al. (1985), and the final preparations were suspended in 10 mM Tris-HCl, pH 7.4, containing either 2% or 30% SDS.

Column Chromatography and Gel Electrophoresis. Porin from strain PLB3255 was applied to a Sephadex G-200 gel filtration column (2.6 × 100 cm) and eluted either with 10 mM Tris-HCl, 0.5% SDS, and 0.02% NaN_3, pH 8.0, or with 10 mM Tris-HCl, 0.5% NaDOC, 0.2 M NaCl, 0.02% NaN_3, and 1 mM EDTA, pH 8.0. Protein absorbance of the eluted fractions was monitored at 280 nm. Fractions were analyzed by SDS–PAGE in a discontinuous buffer system (Laemmli, 1970). Separating gels contained 11% acrylamide and 0.1% SDS with or without 8 M urea. Molecular weight markers included bovine serum albumin (66K), egg albumin (45K), glyceraldehyde-3-P dehydrogenase (36K), carbonic anhydrase (29K), trypsinogen (24K), trypsin inhibitor (20.1K), and α-lactalbumin (14.2K). Protein was detected either by a glutaraldehyde–silver-staining method (Oakley et al., 1980) or by Coomassie Brilliant Blue staining.

Bilayer Lipid Membrane Analysis. To prepare porins for BLM analysis, the proteins were precipitated with 10 volumes of ice-cold 90% acetone (v/v). The protein was centrifuged at 7700*g* for 20 min at 4 °C and washed twice with 90% acetone to remove excess detergent. The porin was then suspended in 1% octyl glucoside and diluted to the appropriate concentration (1–10 μg/mL) for BLM analysis. Membranes were formed by applying a solution of oxidized cholesterol and egg phosphatidylcholine (1:1 w/w) in *n*-decane to a hole in a Teflon partition. This partition separated two chambers containing an aqueous solution of 0.5 M NaCl, pH 5.7. The membranes thinned spontaneously until they formed an optically black bilayer. Silver-silver chloride electrodes (Microelectrodes Inc.) were placed in both sides of the chamber, and a constant voltage was applied across the membrane using a 1.5-V battery. A small amount of porin (20–60 ng/mL) in octyl glucoside was then added to one side of the membrane. Stepwise conductance fluctuations were monitored with a chart recorder using a Keithley Model 614 electrometer for signal amplification.

Liposome Swelling Assay. The liposome swelling assays were performed essentially as described previously (Nikaido & Rosenberg, 1983). Briefly, approximately 6.2 μmol of acetone-extracted egg phosphatidylcholine and 0.2 μmol of dicetyl phosphate were dried onto the bottom of a test tube with a stream of nitrogen. The film was suspended in 0.2 mL of solution containing 20 μg of purified porin suspended in 0.1% octyl glucoside or a solution of 0.1% octyl glucoside without porin for control experiments. The suspension was sonicated in a bath sonicator until the suspension became translucent. The suspension was then dried in a vacuum over $CaSO_4$ overnight. The film was suspended in 0.4 mL of a solution containing 12 mM stachyose, 4 mM sodium NAD, and 1 mM imidazole–NAD buffer, pH 6.0. The suspension was left at room temperature without mixing for 2 h and then shaken by hand. The liposome suspension was passed through an 8-μm nucleopore filter to remove large aggregates, and 40 μL of the suspension was diluted into test solutions. The rate of swelling at 25 °C was monitored by measuring light scattering at 400 nm with a Gilford Response II spectrophotometer. The test solutions contained 1 mM Na-NAD, 1 mM imidazole–NAD buffer, pH 6.0, and one of the following sugars: D-glucose, D-mannose, D-arabinose, D-galactose, sucrose, or lactose, at a concentration of 18 mM.

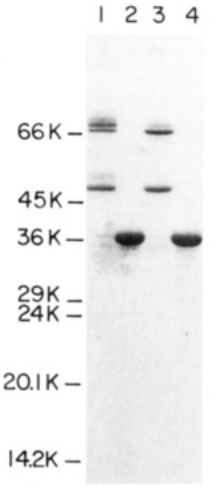

FIGURE 1: Separation of porin from strain PLB3255 on a 12% SDS–polyacrylamide gel stained with Coomassie Brilliant Blue. Lanes 1 and 3 contain porins kept at room temperature before application to the gel. Sample in lanes 2 and 4 were heated for 3 min at 100 °C before application to the gel. Lanes 1 and 2 contain crude porin preparations before separation on a Sephadex G-200 gel filtration column. Lanes 3 and 4 contain LPS-depleted porin recovered after fractionation on a Sephadex G-200 column eluted with 0.5% SDS. Molecular weight markers are those described under Materials and Methods.

Circular Dichroism Spectroscopy. Circular dichroism measurements were made with a JASCO J-500C spectropolarimeter with a 0.5-mm path-length quartz cell. Porin samples at 0.2 mg/mL were suspended in 1% SDS/10 mM sodium phosphate, pH 7.0, and scanned from 350 to 190 nm. The spectra were obtained by subtracting the buffer blank. All measurements were performed at room temperature.

Miscellaneous Chemical Assays. Protein was quantitated by using the BCA protein assay (Pierce Chemical Co.).

Results

Isolation of PLB3255 Porin. Porins were isolated from *E. coli* K-12 strain PLB3255, which produces a mutant OmpF protein (15 amino acid deletion, Δ114–129) and a wild-type OmpC protein (Benson & Decloux, 1985). When the unheated samples were separated by SDS–PAGE and the gel scanned using a Bioimaging scanning densitometer, approximately 50–60% of the isolate migrated as a trimer (72 kDa) with a typical ladder pattern indicating the presence of tightly bound LPS. In addition, between 35% and 40% of the protein migrated as several closely spaced bands with apparent molecular weights of approximately 50K. The remaining 10–15% migrated as proteins of between 35K and 37K molecular weight (Figure 1, lane 1). After the isolate was heated to 100 °C, the trimers and the 50-kDa bands collapsed into a single band at 36 kDa (Figure 1, lane 2). To remove excess LPS, the unheated protein was passed through a Sephadex G-200 gel filtration column, eluted with 0.5% SDS, and concentrated

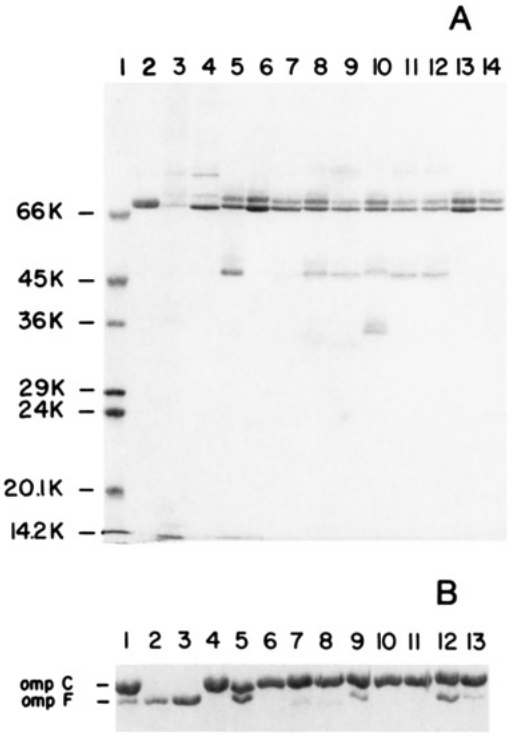

FIGURE 2: (A) 11% SDS–polyacrylamide gel of crude porin isolates from several *E. coli* K-12 strains described under Materials and Methods (and stained with Coomassie Brilliant Blue). Lane 1 contains the molecular weight markers. Lane 2 contains porin from strain D21f2, an Re LPS chemotype. Lanes 3 and 4 contain porin from JF733, which only produces the OmpF porin. Lanes 5, 11, and 12 contain porin from strain PLB3255. Lane 6 contains porin from strain PLB3256. Lane 7 contains strain PLB3252 porin; lane 8, OC200 porin; lane 9, OC208 porin; lane 10, OC209 porin; lanes 13 and 14 contain porin from strain ECB5084. (B) Separation of crude porin isolates on an 11% SDS–polyacrylamide gel containing 8 M urea stained with Coomassie Brilliant Blue. The porin preparations were heated to 100 °C for 3 min before application to the gel. Lane 1 contains porin from strain D21f2. Lanes 2 and 3 contain strain JF733 porin; lanes 4, 10, and 11, PLB3255 porin; lane 5, PLB3256 porin; lane 6, PLB3252 porin; lane 7, OC200 porin; lane 8, OC208 porin; lane 9, OC209 porin; lanes 12 and 13 contain strain ECB5084 porin. Only the relevant portion of the gel is shown.

in an Amicon PM-10 concentrator. The concentrated protein peak when run on an SDS gel showed a reduction in the number of bands in the trimer "ladder" as well as in the 50-kDa aggregate, indicating removal of LPS (Figure 1, lane 3). After the LPS-depleted sample was heated to 100 °C, both aggregates were again resolved as a single band at 36 kDa (Figure 1, lane 4).

Porin Isolation from ompF(dex) and ompC(dex) Deletion and Point Mutants. To determine if other *E. coli dex* mutant strains produced this unique 50-kDa protein aggregate, a set of strains containing either a deletion or a point *dex* mutation in the *ompF* or *ompC* structural gene was examined (Misra & Benson, 1988; Benson et al., 1988). The strains PLB3252, OC200, OC208, and OC209 contained different *ompF(dex)* deletion mutations. Strain PLB3256 contained an *ompC(dex)* deletion mutation, and strain ECB5084 contained an *ompF-(dex)* point mutation. Porins were isolated from each of these strains and characterized by SDS–PAGE. The porin isolated from the five *ompF(dex)* deletion mutants all contained the 50-kDa aggregate (Figure 2A, lanes 5 and 7–10). However, the *ompF* point mutant, ECB5084, did not produce the 50-kDa complex, further indicating that dimers are not forming the expanded channel (Figure 2A, lanes 13 and 14). In addition, strain PLB3256, carrying an *ompC(dex)* deletion mutation and the wild-type OmpF porin gene, did not produce a 50-kDa

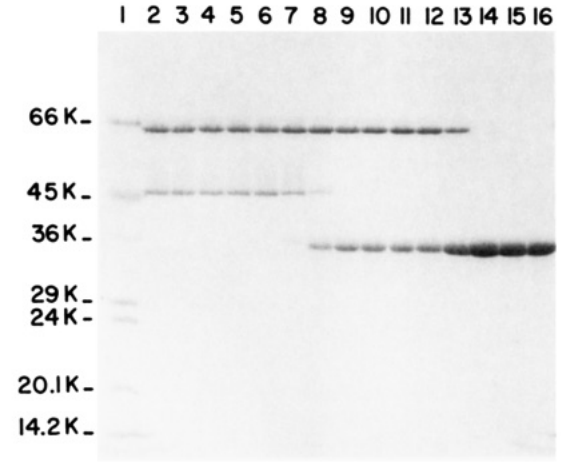

FIGURE 3: Temperature stability of LPS-depleted porin isolated from strain PLB3255. The porin was heated at different temperatures for 5 min and then applied to a 12% SDS–polyacrylamide gel. The gel was stained with Coomassie Brilliant Blue. Lane 1 contains the molecular weight markers. Lane 2 contains sample heated at 25 °C, lane 3 at 37 °C, lane 4 at 45 °C, lane 5 at 50 °C, lane 6 at 55 °C, lane 7 at 60 °C, lane 8 at 63 °C, lane 9 at 66 °C, lane 10 at 69 °C, lane 11 at 72 °C, lane 12 at 75 °C, lane 13 at 78 °C, lane 14 at 81 °C, lane 15 at 85 °C, and lane 16 at 100 °C.

complex. These porin isolates were also heated and run on an 11% SDS gel containing 8 M urea. The major band detected in the isolates from all of the *ompF(dex)* deletion mutants migrated identical with wild-type OmpC porin (Figure 2B, lanes 4 and 6–9). The mutant *ompF(dex)* gene product was not recovered in large amounts from any of these strains except OC209 (Figure 2B, lane 9). Porins from both the wild-type *ompF* and the *ompC(dex)* genes were recovered from strain PLB3256 in nearly equal amounts (Figure 2B, lane 5). Similarly, high amounts of the mutant *ompF* gene product were isolated from the peptidoglycan of the *ompF(dex)* point mutant ECB5084 (Figure 2B, lanes 12 and 13). Since the 50-kDa OmpC aggregate was isolated in the highest amounts in strain PLB3255 with the smallest contamination by the mutant OmpF protein, further characterization of the OmpC dimer was performed by using this strain.

Temperature Stability of the 50-kDa Dimers. LPS-depleted porin, following Sephadex G-200 purification of the PLB3255 isolate, was used to determine the heat stability of the trimer and the 50-kDa dimer. The results showed that the 50-kDa complex, when heated in SDS, is less stable than the trimer and denatures between 60 and 66 °C (Figure 3, lanes 7–9). The heat stability of the OmpC trimer in SDS is slightly higher than that of the wild-type OmpF trimer of *E. coli* (data not shown) and denatured only above 78 °C (Figure 3, lanes 13 and 14). In addition, removal of excess LPS from the OmpC dimers and trimers did not affect their heat stabilities (data not shown). A 50-kDa OmpF dimer, shown to be an intermediate in the assembly of wild-type trimers, has also been reported to denature at lower temperatures, i.e., between 45 and 58 °C (Reid et al., 1988).

Dimer Purification by Gel Filtration Chromatography in Sodium Deoxycholate. Dimer and trimer aggregates of OmpC porin, isolated from strain PLB3255, were solubilized in 30% SDS, applied to a Sephadex G-200 column, and eluted with 0.5% sodium deoxycholate. This column removed large porin aggregates and excess LPS. The majority of the protein was recovered in two distinct peaks, corresponding to the OmpC trimer and OmpC dimer (data not shown). When the column fractions were run on SDS–PAGE, the leading edge of the first peak contained purified OmpC trimers, and the trailing edge of the second peak contained purified dimers

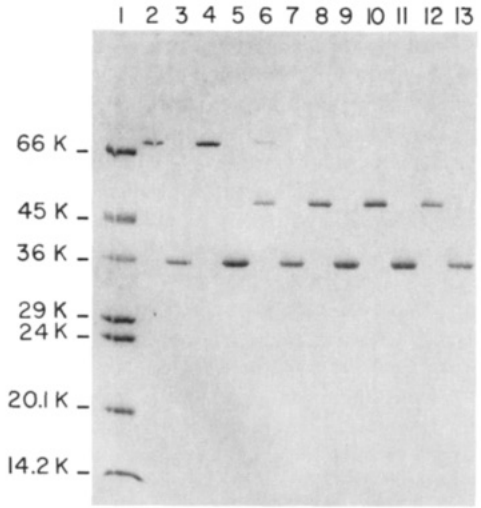

FIGURE 4: A 12% SDS–polyacrylamide gel of fractions of PLB3255 porin eluted from a Sephadex G-200 column with 0.5% sodium deoxycholate/10 mM Tris pH 8.0. The samples in lanes 2, 4, 6, 8, 10, and 12 were left at room temperature before application to the gel. Samples in lanes 1, 3, 5, 7, 9, 11, and 13 were heated to 100 °C for 3 min before application to the gel. Lanes 2 and 3 contain column fraction 43, lanes 4 and 5 contain fraction 45, lanes 6 and 7 contain 47, lanes 8 and 9 contain fraction 49, lanes 10 and 11 contain fraction 50, and lanes 12 and 13 contain fraction 51. The gel was stained with Coomassie Brilliant Blue.

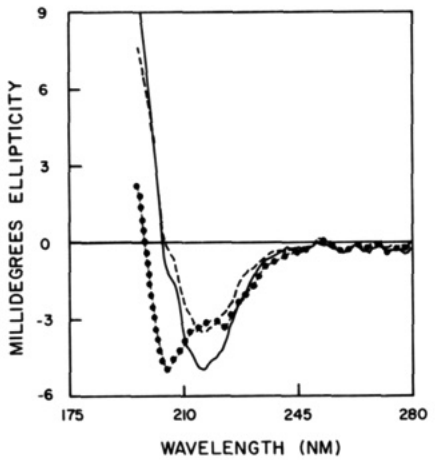

FIGURE 5: Circular dichroism spectra of OmpC dimers (---), trimers (—), and monomers obtained by heating OmpC trimers (●—●). Samples at 0.2 mg/mL were suspended in 10 mM sodium phosphate, pH 7.0, containing 1% SDS.

(Figure 4). These results further indicate that the dimer is a smaller complex of the OmpC porin than the trimer isolate. That the 50-kDa bands migrate in SDS gels midway between the trimer and the monomer also suggests that the protein is a dimer of porin.

Circular Dichroism Spectra of Dimers and Trimers. The secondary structures of the OmpC dimer and trimer isolates were determined by measuring their circular dichroism spectra. The spectra of the two isolates were similar in shape, and both exhibited a minimum ellipticity at 216 nm with a crossover of 204 nm for the trimer and 205 nm for the dimer (Figure 5). The spectra indicate that both dimers and trimers contain a large amount of β-structure similar to what has been reported by others (Rosenbusch, 1974; Markovic-Housley & Garavito, 1986). The spectrum of the trimer sample, after being heated to 100 °C for 2 min, showed a loss of β-structure and an increase in α-helix and random coil.

Assays of Porin Activity. To measure the channel-forming activity, the isolated porin complexes were reconstituted into two different artificial membrane systems, liposomes and planar BLM's. The liposome swelling assay that was used is outlined under Materials and Methods (Nikaido & Rosenberg, 1983). The rate of liposome swelling was followed spectrophotometrically for 3 min for monosaccharides and between 20 and 180 min for disaccharides. Solutes unable to penetrate the porin channels caused no change in the osmotic equilibrium, and no swelling was observed. This was also true for control liposomes without porin and for liposomes containing non-porin proteins. It is assumed that the initial rate of uptake of solutes into the liposomes, i.e., the initial rates of optical density decrease, reflects the solute penetration rate into the outermost layer of the liposome (Nikaido & Rosenberg, 1983). The results in Table I show that both the dimer and the trimer allowed all solutes tested to enter the liposome; not surprisingly, the large disaccharides, sucrose and lactose, diffused at a much slower rate than did the monosaccharides. In addition, the relative diffusion rates and size-exclusion limits of the isolated dimers and the trimers were similar; therefore, the two aggregates appear to have a similar channel size. SDS-solubilized liposomes containing either the OmpC dimers or the trimers were applied to SDS–polyacrylamide gels, and their mobility was measured to determine whether the dimers (and trimers) maintained their aggregate nature in the liposomes used in the swelling assay. The results in Figure 6 show each of the porins tested maintained their aggregate nature when placed into phospholipid liposomes (lanes 3 and 4). The slight contamination of trimer in the dimer-containing liposomes (lane 4) was present in the dimer isolate prior to liposome incorporation and does not appear to be significant.

The results from the BLM analysis of dimer and trimer isolates also indicated that both porin samples had channel-forming activity. Stepwise current increases and decreases across the bilayer membranes in the presence of OmpC dimers and trimers were analyzed at a membrane potential of 25 mV. The conductance change across the membrane, Λ, was measured and divided by the specific conductance of the 0.5 M

Table I: Diffusion Rates of Saccharides through Porin Channels[a]

solutes	mol wt	initial swelling rates of liposomes containing[b] PLB3255 dimer	PLB3255 trimer
D-arabinose	150	0.96	0.97
D-mannose	180	1.0	1.0
D-galactose	180	0.88	0.88
D-glucose	180	0.90	0.94
lactose	342	0.08	0.11
sucrose	342	0.04	0.03

[a] In each experiment, 20 μg of protein was added to liposomes as outlined under Materials and Methods. [b] The swelling rates were normalized to the fastest permeating sugar, D-mannose.

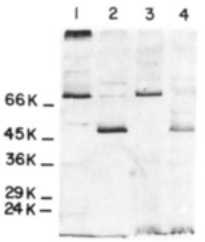

FIGURE 6: A 12% SDS–polyacrylamide gel of isolated OmpC dimers and trimers from strain PLB3255 before and after addition to liposomes. Lanes 1 and 3 contain porin trimers. Lanes 2 and 4 contain OmpC dimers. Lanes 1 and 2 contain porins that were used to form liposomes. Lanes 3 and 4 contain the porins recovered from liposomes, by solubilization in 4% SDS prior to electrophoresis. The gels were silver stained as described under Materials and Methods.

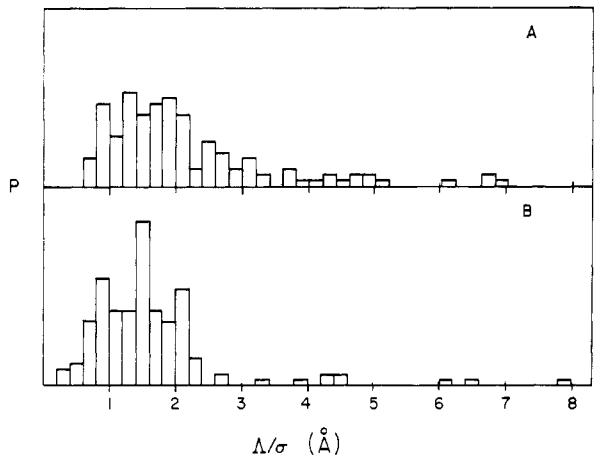

FIGURE 7: Distribution of the size parameter, Λ/σ (Å), for porin trimer (A) and dimer (B) isolates of strain PLB3255 measured in BLM's. The porin at 50 ng/mL was added to the bathing solution. Electrical conductance across the membrane was measured by using a transmembrane potential of 25 mV. Λ is the conductance change, and σ is the specific conductance of the bathing solution. *P*, in arbitrary units, is the relative number of events with the given size parameter values. Purified trimer and dimer were isolated from a Sephadex G-200 gel filtration column eluted with 0.5% NaDOC. The isolates were resuspended in 0.1% octyl glucoside after acetone precipitation. Both opening and closing events are included in the histogram.

NaCl bathing solution, σ. In this study, the conductance changes are reported as a size parameter, Λ/σ, in angstroms, plotted as a function of the probability, *P*, of a channel of a given size. The size distribution histogram was then derived for each porin isolate (Figure 7). The results indicate that the channel sizes were similar for the two porins; 134 events were observed for the OmpC trimer and 152 events for the OmpC dimer. The average specific conductance for the trimer was 1.78 ± 0.73 Å whereas for the dimer this measurement was 1.43 ± 0.57 Å.

Discussion

E. coli K-12 strain PLB3255 can grow on maltodextrins in the absence of a LamB porin due to a mutation (*dex*) that was mapped to the *ompF* gene (Benson & Decloux, 1985). The mutation was shown to result in an OmpF protein with a deletion of 15 amino acid residues (Benson et al., 1988). Supposedly, the deletion increases the size of the porin channel. Presumably, the PLB3255 strain can grow on maltodextrins because the mutant OmpF(dex) porin is functional in the outer membrane. However, the OmpF(dex) porin appears unable to form stable trimers and may be functional either as unstable trimers or as monomers. When we set out to examine this OmpF(dex) porin from strain PLB3255, we found that the mutant OmpF porin did not associate tightly with the peptidoglycan layer and could not be recovered in large amounts using standard protocols. The 15 amino acid deletion in the polypeptide chain appears to alter the peptidoglycan–OmpF porin interactions. In addition, we observed a typical 72-kDa trimer and an unusual 50-kDa "dimer" in the unheated porin isolates of this strain which travelled as a 36-kDa polypeptide when heated to 100 °C. Careful analysis of the 50-kDa protein indicated that it was an aggregate of the wild-type OmpC porin.

Surprisingly, we found that all of the *ompF*(*dex*) deletion mutant strains produced the 50-kDa aggregate. Furthermore, the larger the deletion in the OmpF protein, the lower the amount of mutant OmpF porin that was recovered on the peptidoglycan and the greater the level of OmpC "dimer" that was isolated. All of these deletions changed the overall charge of the mutant OmpF porin due to the loss of one or more aspartic acid residues. Perhaps this alteration in charge modified the OmpF conformation and interaction with components such as the peptidoglycan or other porin subunits, in turn affecting the assembly of other outer membrane proteins. The *ompF* point mutant, ECB5084, which also produced a larger pore, contained no detectable dimers, and while this mutant porin lost a negatively charged aspartic acid residue, which was substituted by glycine, this mutant porin bound tightly to peptidoglycan. Thus, loss of this single negatively charged amino acid does not appear to be critical in the assembly of the trimer or binding of the porin to peptidoglycan. It is known that the electrochemical potential of the inner membrane, the signal sequence of the secreted protein, and the three-dimensional protein configuration are important factors in determining the final destination of outer membrane proteins. Altering one or more of these essential determinants could jam the assembly and export of porin. Mutant OmpF(dex) porin, containing the same 15 amino acid deletion as the OmpF porin in PLB3255, produced by a strain lacking an *ompC* gene does not form stable trimers or dimers in the presence of SDS (data not shown). This mutant OmpF porin also does not bind well to the peptidoglycan. This mutation in the *ompF* gene in the PLB3255 strain could be affecting the aggregation of the wild-type OmpC porin by jamming porin assembly, resulting in the accumulation of dimer intermediates which are exported to the outer membrane. Surprisingly, the *ompC*(*dex*) mutation in strain PLB3256 did not alter the OmpF porin assembly. This suggests that the OmpC and OmpF porins may have different assembly/export pathways. Either the OmpC(dex) porin adopts a different conformation than the analogous OmpF(dex) porins or they are recognized differently by assembly/transport systems.

Another explanation for the production of OmpC dimers in *ompF*(*dex*) deletion mutants could be that the dimer is a result of the breakdown of a heterotrimer consisting of two wild-type OmpC subunits and a single mutant OmpF polypeptide. Heterotrimers have been shown to exist in cross-linking studies between OmpC and OmpF monomers (Ichihara & Mizushima, 1979). Because of the altered structure of the OmpF porin, the heterotrimer might readily break down to OmpF monomer and a stable OmpC dimer. At this time, we have no strong evidence to support either model for OmpC dimer formation.

In our initial characterization of the OmpC dimer, we found that this aggregate is a stable porin complex that is tightly associated with the peptidoglycan. Its secondary structure appears to be very similar to that of the trimer. The OmpC dimer is not the component which confers on the PLB3255 cells the ability to grow on maltodextrin or to have dye and detergent sensitivity since the mutant *ompF*(*dex*) strains lacking an *ompC* gene have the ability to grow on maltodextrins and have similar dye and detergent sensitivities. Upon analyzing the functioning of the 50-kDa aggregate, we found that the dimer has a channel size similar to that of the wild-type OmpC trimer. It is not known if the dimer is functional on the outer surface of the intact cell. Yet, the PLB3255 cells grow at a rate similar to wild-type *E. coli* K-12 cells. So perhaps the dimers, comprising 35–40% of the total ompC porin, are active in forming channels in intact cells.

The OmpC dimer when reconstituted into liposomes and BLM's appeared to have channel properties similar to that of the trimer, but there are some differences between the two isolates. One important difference, other than their mobilities on SDS–PAGE, may be the amount and affinity of LPS bound

to each. Crude wild-type OmpF porin from *E. coli* K-12 strain JF733 forms a typical ladder pattern on SDS–PAGE, indicating the presence of different amounts of bound LPS (Rocque et al., 1987). Most of this LPS can be removed by solubilizing the porin in high detergent concentrations, e.g., 30% (w/v) SDS. Preliminary evidence suggests that the 50-kDa dimer either binds less LPS than trimer, since fewer bands were seen, or binds LPS less tightly, since the multiple bands of the dimer ladder pattern disappeared more readily during the purification on Sephadex G-200. LPS bound to porin may have several critical roles including stabilization of porin structure, regulation of porin activity (Schindler & Rosenbusch, 1978, 1981), and contribution to the structure necessary for bacteriophage recognition (Mutoh et al., 1978; Henning & Jann, 1979). We have found that removal of excess LPS from porin dimers and trimers does not affect their heat stabilities. However, little is known about LPS interactions which may be necessary for porin assembly and export to the outer membrane. Perhaps LPS is essential for the porins to be properly folded and transported to the outer membrane. It has been suggested that LPS influences the rate of oligomerization and porin insertion into the outer membrane (Reid et al., 1988).

Previously, the trimer was thought to be the smallest functional unit of *E. coli* porins (Nakae et al., 1979). When chemical cross-linking is used, porin protein F from *Pseudomonas aeruginosa* has been shown to exist as a trimer in the outer membrane (Angus & Hancock, 1983), but upon purification, this aggregate breaks down into monomers that are still functionally active in planar bilayer membranes (Woodruff et al., 1986) and liposomes (Yoshimura et al., 1983). In addition, porin from *Rhodopseudomonas sphaeroides* exists as a functionally active oligomer of 67-kDa, but upon addition of EDTA or heat, the porin breaks down to functionally active "monomers" of 47 kDa which retain their secondary structure (Weckesser et al., 1984). Unlike the porins from *Pseudomonas* and *Rhodopseudomonas*, isolated porin monomers of *E. coli* are not functional. However, within trimeric complexes, each monomer appears to contain a diffusion channel (Xu et al., 1986).

In conclusion, the results presented here show that the strains carrying *ompF(dex)* deletion mutations and a *ompC*-(wt) gene are altered not only in the structure of the OmpF protein but also in the assembly of OmpC subunits. These strains appear to produce OmpC dimers which accumulate and are transported to the outer membrane. These dimers are stable and exhibit channel-forming activity in reconstituted membrane systems. We are presently attempting to determine if these *ompF(dex)* deletion mutations can also alter the assembly of other outer membrane porin proteins.

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Registry No. D-Arabinose, 10323-20-3; D-mannose, 3458-28-4; D-galactose, 59-23-4; D-glucose, 50-99-7; lactose, 63-42-3; sucrose, 57-50-1.

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