JOURNAL OF BACTERIOLOGY, Apr. 1994, p. 2259–2264
0021-9193/94/$04.00+0

Vol. 176, No. 8

Assembly of LamB and OmpF in Deep Rough Lipopolysaccharide Mutants of *Escherichia coli* K-12

MICHAEL W. LAIRD, ANDREW W. KLOSER, AND RAJEEV MISRA*

Department of Microbiology, Arizona State University, Tempe, Arizona 85287-2701

Received 9 November 1993/Accepted 6 February 1994

Assembly of the OmpF and LamB proteins was kinetically retarded in deep rough lipopolysaccharide mutants of *Escherichia coli* K-12. OmpF assembly was affected at the step of conversion of metastable trimers to stable trimers, whereas LamB assembly was influenced both at the monomer-to-metastable trimer and metastable-to-stable trimer steps. These assembly defects were reversed in the presence of the *sfaA1* and *sfaB3* suppressor alleles, which were isolated by using *ompF* assembly mutants.

The cell envelope surrounding an *Escherichia coli* K-12 cell is a complex structure composed of inner and outer membranes with an aqueous periplasmic space sandwiched between these membranes. The outer membrane is composed of proteins, lipids, and lipopolysaccharide (LPS). LPS is found almost entirely within the external leaflet of the outer membrane (17), where it serves as an important component of the permeability barrier on the exterior of gram-negative bacteria (19). LPS is closely associated with the outer membrane proteins OmpF and LamB (1, 7). These trimeric proteins, also known as porins, form water-filled channels to allow diffusion of substrates into the cell (3). It has been shown that deep rough mutants, which lack part of the LPS inner core, possess greatly reduced amounts of porin proteins (1, 12). In addition, LPS mutants lacking several *rfa* genes that are responsible for the synthesis of LPS outer core and for phosphorylating the heptose residues of the inner core produced reduced levels of porins (20). Biochemical studies from Nikaido's laboratory showed the in vitro role of LPS during the trimerization of OmpF (27, 28). It has also been shown that in vitro, these proteins can be trimerized in the absence of LPS (8).

OmpF is initially synthesized as a precursor containing a 22-amino-acid signal sequence at the amino-terminal end. Cleavage of the signal sequence at the periplasmic face of the inner membrane results in the release of mature monomers (2). Prior to assembling into heat-stable trimers, these monomers form heat-labile dimers and metastable trimers (9, 23). The site of OmpF trimerization remains unclear. The heat-stable form of trimer functions as a nonspecific channel through the outer membrane (18) and serves as the receptor for bacteriophage K20 (30).

Similar to the case with OmpF, LamB monomers have been shown to form metastable trimers before assembling into stable trimers. However, in the case of LamB, a dimeric assembly intermediate has not been reported (32). It has been previously shown that during LamB assembly, mature monomers are targeted to the outer membrane, where they are then able to trimerize (16). This process was observed by using a mutant LamB protein in which the trimerization process could be conditionally blocked. Stable LamB trimers form water-filled pores that facilitate diffusion of maltose and maltooligomers (31) in addition to serving as the receptor for the bacteriophage λ (6, 21).

* Corresponding author. Phone: (602) 965-3320. Fax: (602) 965-0098. Electronic mail address: AARXM@ACVAX.INRE.ASU.EDU.

In this study, we were interested in understanding the in vivo role of LPS during the assembly of OmpF and LamB. This was accomplished by observing the assembly of these porin proteins in strains containing *rfa* deletions which produced truncated LPS molecules lacking the outer core. In addition, these deletions removed the *rfaP* gene, whose product is reported to be involved in the phosphorylation of the heptose residues of the inner core (25). We have shown that in these deep rough LPS mutants, the kinetics of porin assembly were considerably retarded compared with the rate observed in the wild-type strain. We have also demonstrated the interaction point(s) of LPS in the assembly processes of OmpF and LamB. In addition, we present results showing that the defective porin assembly observed in deep rough mutants could be alleviated by *sfaA1* or *sfaB3*, two extragenic suppressors isolated by using OmpF assembly mutants.

MATERIALS AND METHODS

Media and chemicals. Minimal medium (M63) and Luria broth were prepared as previously described by Silhavy et al. (29). When cells were grown on minimal medium, 0.4% glycerol was used as the sole carbon source. [^{35}S]methionine was purchased from Du Pont-New England Nuclear. All other chemicals were of analytical grade.

Bacterial strains and genetic techniques. The strains used in this study were constructed in an MC4100 background (5). The two *rfa* deletions, Δ*rfa-2057* and Δ*rfa-2058*, were moved by P1 transductions using chloramphenicol-resistant (Cm^r) markers linked to the deletions. These deletions were moved from CAS2057 and CAS2058, respectively (20). Strains containing *rfa* deletions were checked for resistance to the bacteriophage U3, which utilizes wild-type LPS as the receptor. These strains were transduced with *cps*::Tn*10* or *rcsB*::Kan^r because the deletion of the *rfa* locus resulted in the overproduction of capsule. In addition, all strains used here were transduced with an *ompC*::*lacZ* fusion. The removal of a functional *ompC* gene was necessary to avoid cross-reactivity of OmpF antiserum with OmpC.

To study the effects of the suppressor alleles (*sfaA1* and *sfaB3*) on the assembly of OmpF and LamB in a *rfa*$^+$ or Δ*rfa* background, strains were constructed in the following manner. A strain carrying an *ompC*::*lacZ* fusion was first transduced with a *cps*::Tn*10* or *rcsB*::Kan^r marker linked to either a suppressor or wild-type allele of *sfaA*. *sfaB* alleles were moved by using a linked *leu*::Tn*10* marker. The presence of the suppressor allele of *sfaA* or *sfaB* was confirmed by marker

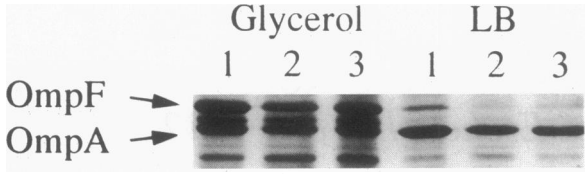

FIG. 1. SDS-PAGE analysis of envelopes prepared from wild-type and mutant LPS strains. Strains were grown overnight at 37°C in either glycerol minimal media or Luria broth (LB). Lane 1, wild type (*rfa*$^+$); lane 2, Δ*rfa-2057* mutant; lane 3, Δ*rfa-2058* mutant. The positions of OmpF and OmpA are shown.

rescue using a strain that contains a mutant OmpF protein (15). Proper assembly and thus a Dex$^+$ phenotype of this mutant protein are dependent on the suppressor allele, *sfaA1* or *sfaB3*. The *rfa* deletions were then transduced into strains carrying either the wild-type or suppressor allele of *sfaA* or *sfaB* by using a linked Cm^r marker. P1 transductions were performed as described previously (29).

Pulse-chase experiments and immunoprecipitation. Pulse-chase experiments were performed on cells grown to mid-log phase in 0.4% glycerol minimal medium (16). Extraction of radioactively labeled trimers was completed as described previously (15). Trimers were immunoprecipitated from cell extracts with appropriate antibodies. The immunocomplexes were precipitated by formalin-killed *Staphylococcus aureus* cells. These immunoprecipitates were washed several times and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The gels were dried and fluorographed at −70°C. Protein bands were quantified with a Hoefer GS300 scanning densitometer.

SDS-PAGE of cell envelopes. Envelopes from cells grown overnight were prepared by the French press lysis procedure as described previously (16). Samples were electrophoresed on SDS-polyacrylamide (11%, 4 M urea) gels (13) and stained with Coomassie blue.

RESULTS

Construction and characterization of LPS mutant strains. To study the effect of mutant LPS on the assembly of porin proteins, two chromosomal deletions removing part of the *rfa* locus were transduced into an MC4100 genetic background. The *rfa* locus encodes enzymes required for LPS core synthesis. The deletion Δ*rfa-2057* removes 5.0 kb of DNA extending from the 5′ end of *rfaG* through the 3′ end of *rfaI* and results in truncated LPS molecules containing two unphosphorylated heptose residues and no hexose residues (20). The second deletion, Δ*rfa-2058*, is slightly larger than Δ*rfa-2057*, extends from the 5′ end of *rfaQ* through the 3′ end of *rfaJ*, and results in a truncated structure which could not be differentiated from that produced by Δ*rfa-2057* (20). Strains carrying these two deletions were similar in their resistance to the bacteriophage U3 and sensitivity to hydrophobic antibiotics, namely, novobiocin and rifampin. We examined the envelopes prepared from strains carrying either wild-type or defective LPS in two different growth media. When these strains were grown in glycerol minimal medium, no significant effect of the mutant LPS molecules on the levels of OmpF and OmpA was observed (Fig. 1). However, growth in Luria broth greatly reduced OmpF levels, but not the levels of OmpA, in cells carrying the defective truncated LPS. These LPS mutant strains became resistant to the OmpF-specific bacteriophage K20. Quantification of this effect revealed a 10^2- to 10^3-fold reduction in the efficiency of plaquing for K20 on the *rfa* deletion strains (Table 1). Furthermore, K20 was unable to form clear plaques on Δ*rfa* strains.

TABLE 1. Phage sensitivity patterns of *rfa*$^+$ and Δ*rfa* strains in various genetic backgrounds.

Strain genotype	Phage sensitivity[a]			K20 EOP[b]
	U3	λ	K20	
rfa$^+$ *sfaA*$^+$	S	S	S	1.0
rfa$^+$ *sfaA1*	S	S	S	1.0
Δ*rfa-2057 sfaA*$^+$	R	S	R	1.1×10^{-2}
Δ*rfa-2057 sfaA1*	R	S	S	0.42
Δ*rfa2058 sfaA*$^+$	R	S	R	5.5×10^{-3}
Δ*rfa-2058 sfaA1*	R	S	S	0.36
rfa$^+$ *sfaB*$^+$	S	S	S	1.0
rfa$^+$ *sfaB3*	S	S	S	1.0
Δ*rfa-2057 sfaB*$^+$	R	S	R	1.5×10^{-3}
Δ*rfa-2057 sfaB3*	R	S	S	0.36
Δ*rfa-2058 sfaB*$^+$	R	S	R	1.6×10^{-2}
Δ*rfa-2058 sfaB3*	R	S	S	0.65

[a] Bacteriophages U3, λ, and K20 utilize complete LPS, LamB, and OmpF, respectively, as their receptors. Phage sensitivity tests were carried out on Luria broth agar. Abbreviations: S, sensitivity; R, resistance. *sfaA*$^+$ and *sfaB*$^+$ are wild-type *sfaA* and *sfaB* alleles. *sfaA1* and *sfaB3* are suppressor (su$^+$) alleles of *sfaA* and *sfaB*, respectively.

[b] EOP, efficiency of plaquing.

Effect of mutant LPS on OmpF trimerization. To observe the assembly of OmpF in LPS mutant backgrounds, trimer assays were performed with strains with intact LPS (*rfa*$^+$) and the two Δ*rfa* strains described above. Proteins were extracted from [^{35}S]methionine-labeled cells and subjected to immunoprecipitation with OmpF trimer-specific antibodies. These immunoprecipitates were heated to 65°C and analyzed by SDS-PAGE followed by fluorography. Heating at 65°C resulted in the dissociation of metastable trimers to monomers. Thus, in all experiments in which trimer-specific antibodies were used, protein molecules migrating at the monomeric position are metastable trimers (see Fig. 2, 4, 8, and 10).

The results of the OmpF trimer assay are shown in Fig. 2. The first six lanes show the assembly of OmpF in an *rfa*$^+$ background, which displayed a 50% trimer-to-monomer ratio at approximately 1 min postchase. In contrast, the two Δ*rfa* strains, shown in the next 12 lanes, exhibited a reduced rate of OmpF trimerization, such that a 50% trimer-to-monomer ratio was not achieved until roughly 10 min postchase. Protein bands

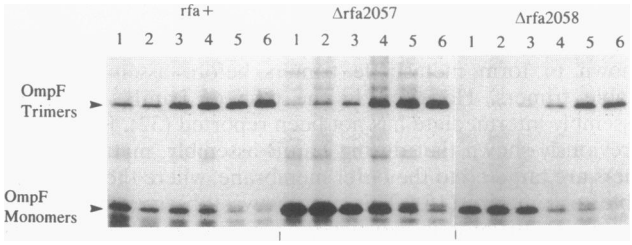

FIG. 2. OmpF trimer assays of wild-type and LPS mutant strains. Cells were grown to mid-log phase in glycerol minimal medium, labeled for 20 s with [^{35}S]methionine, and chased with an excess of nonradioactive methionine. Chase samples were removed after 0.5, 1, 3, 10, 30, and 60 min (lanes 1 to 6, respectively). The samples were lysed, and OmpF was immunoprecipitated with trimer-specific antibodies. Immunoprecipitates were heated to 65°C prior to SDS-PAGE analysis. The gel was dried and subjected to autoradiography. The positions of OmpF trimers and monomers are shown.

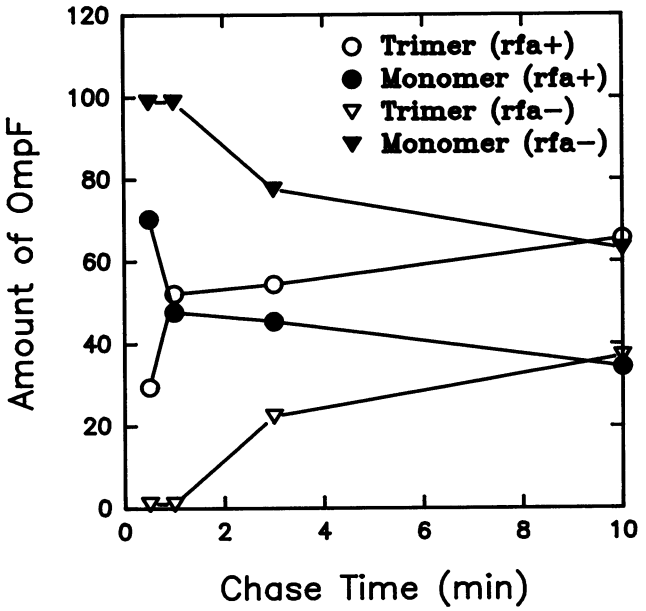

FIG. 3. Quantification of the OmpF trimer assays shown in Fig. 2. Data from the first four chase samples were plotted.

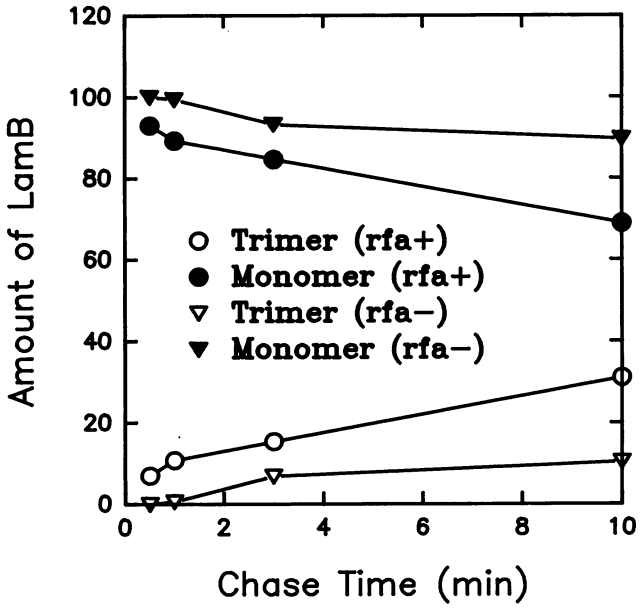

FIG. 5. Quantification of the LamB trimer assays shown in Fig. 4. Data from the first four chase samples were plotted.

were quantified with a densitometer. Only the first 10 min of the quantified data are shown for clarity (Fig. 3). The quantitative data showed that in these LPS mutants OmpF trimerization occurred at a rate significantly slower than that observed in the wild-type strain. Interestingly, after 60 min of chase, the wild-type and mutant strains had the same OmpF trimer-to-monomer ratio present. Thus, it is only the kinetics of trimerization, and not the overall ability to form trimers, that is affected by the mutant LPS. This conclusion is consistent with the results mentioned above (Fig. 1), which showed that under steady-state growth conditions in glycerol minimal medium, similar levels of OmpF were present in *rfa*$^+$ and Δ*rfa* strains.

Effect of mutant LPS on LamB trimerization. Next, we examined the effect of mutant LPS on the trimerization of LamB. This assay was similar to that described for OmpF, and the results are shown in Fig. 4. In LPS mutant strains, LamB trimerization occurred at a reduced rate compared with that in the wild-type strain. The gel was quantified, and the graph is shown in Fig. 5. Note that the effect of LPS is more evident during OmpF trimer assembly than during the assembly of LamB trimers. Again, as was observed with OmpF, only the rate of trimerization, and not the overall ability to form LamB trimers, was influenced in the mutant LPS background.

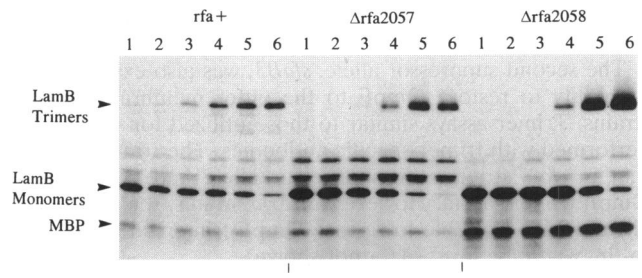

FIG. 4. LamB trimer assays of wild-type and LPS mutant strains. Growth and labeling conditions were similar to those described for Fig. 2. Samples were removed after 0.5, 1, 3, 10, 30, and 60 min of chase (lanes 1 to 6, respectively). The samples were lysed and immunoprecipitated with LamB trimer-specific antibodies as well as maltose-binding protein (MBP) antibodies. Immunoprecipitates were prepared and analyzed similarly to those in Fig. 2. The band positions of the MBP and the LamB trimers and monomers are shown.

Steps at which LPS interacts during porin assembly. In an attempt to understand the assembly pathway and possible interactive points for the effect of LPS on trimerization, we use the simple model shown in Fig. 6. If LPS interaction is required at the step designated A, we would expect to see a buildup of monomeric intermediate forms in the Δ*rfa* strains. However, if LPS is required at the step designated B, we would expect to see a buildup of metastable trimers. In addition, it is conceivable that both steps A and B could be affected by LPS.

To examine these possibilities, we performed trimer assays utilizing monomer- or trimer-specific antibodies for OmpF and LamB, as described in Materials and Methods. As mentioned above, the accumulated OmpF species migrating at the monomeric position is a metastable form of OmpF, which upon heating to 65°C dissociates into monomers (Fig. 2). Although our trimer-specific OmpF antibodies mainly recognize the trimeric species, some monomers are also brought down during immunoprecipitation (data not shown). Thus, the accumulated intermediate in Fig. 2 can either be trimeric (i.e., metastable trimers) or monomeric in nature. To decide between these two possibilities, we utilized OmpF monomer-specific antibodies, which do not recognize trimeric forms. This

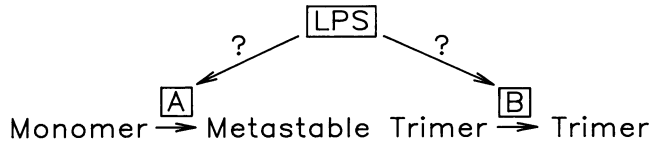

FIG. 6. Model showing the possible steps at which LPS could be involved in porin assembly.

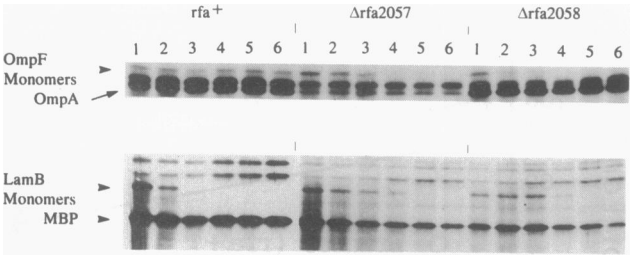

FIG. 7. Assay of OmpF and LamB monomers from strains carrying wild-type or mutant LPS. Growth and labeling conditions were similar to those described for Fig. 2. The samples were lysed and immunoprecipitated with either OmpF monomer-specific antibodies (top gel), or LamB monomer-specific antibodies and maltose-binding protein (MBP) antibodies (bottom gel). OmpF and LamB immunoprecipitates were heated to 100°C prior to analysis by SDS-PAGE (top and bottom gels, respectively). The gels were dried and subjected to autoradiography. The positions of the relevant protein bands are shown. The protein band migrating between OmpF and OmpA is that of OmpT.

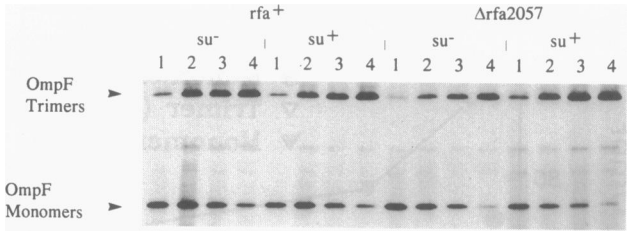

FIG. 8. OmpF trimer assays of wild-type and mutant LPS strains in the presence and absence of the *sfaA* suppressor (su) allele. Growth and labeling conditions were similar to those described for Fig. 2. Chase samples were removed after 1, 5, 10, and 30 min, (lanes 1 to 4, respectively). The samples were lysed, immunoprecipitated with trimer-specific OmpF antibodies, and analyzed similarly to those in Fig. 2. The positions of the relevant protein bands are shown.

experiment showed that in the *rfa*$^+$ strain, a form of OmpF migrating at the monomeric position persisted up to 60 min of chase (Fig. 7, top gel). However, in Δ*rfa* strains, OmpF monomers were not seen after 3 min of chase (Fig. 7, top gel). The monomeric forms of OmpF present in wild-type and mutant strains were not observed when the samples were not boiled prior to SDS-PAGE analysis (data not shown). We believe that the monomeric form seen in the later chase samples in the wild-type strain is not kinetically related to OmpF trimer assembly. Thus, it appears likely that in mutant LPS backgrounds, OmpF assembly is affected at step B, the conversion from metastable to stable trimers. This defect leads to a buildup of metastable trimers and not monomers (Fig. 2).

To examine the step at which LamB assembly was affected in the mutant LPS backgrounds, experiments similar to those described for OmpF were performed. The use of monomer-specific LamB antibodies showed that true monomers persisted for at least up to 10 min in LPS mutant strains, whereas in the wild-type strain monomers were observed for only up to 1 min (Fig. 7, bottom gel). This suggested that LamB assembly was affected by LPS at step A, during the conversion of monomers to metastable trimers. In addition, it appears that step B, involving conversion of metastable to stable trimers, is affected by LPS (Fig. 4). In the wild-type strain, a significant amount of stable trimers appeared at roughly 3 min postchase. However, in LPS mutant strains, almost no stable trimers were observed after 3 min of chase. Instead, a large quantity of metastable trimers accumulated, which migrate at the monomeric position under these assay conditions. These results showed that both steps A and B, involving conversion of monomers to metastable trimers and of metastable to stable trimers, were affected in mutant LPS backgrounds.

Suppression of the LPS-mediated assembly defect. In this portion of the study, we examined whether it was possible to reverse the LPS-mediated assembly defect by using the mutant alleles of *sfaA* and *sfaB* which were isolated as suppressors of *ompF* assembly mutants (11, 15). These genes map at 45 and 2 min, respectively, on the *E. coli* chromosome. Genetic and biochemical experiments showed that these suppressors permitted the assembly of certain mutant OmpF proteins (11, 15).

The suppressor alleles of *sfaA* and *sfaB* were brought into strains carrying *rfa* deletions by P1 transductions using linked markers. As a control, these alleles were also transduced into the wild-type (*rfa*$^+$) background. The presence of the suppressor alleles was confirmed by marker rescue experiments (see Materials and Methods).

As mentioned above, simple phage cross-streak experiments showed that the Δ*rfa* strains were resistant to the OmpF-specific bacteriophage K20. However, in the presence of either suppressor allele, the LPS mutant strains became sensitive to K20. Thus, the efficiency of plaquing for K20 in the presence of either *sfaA1* or *sfaB3* was similar to that seen in the wild-type strain (Table 1). However, these Δ*rfa* strains remained sensitive to λ in the presence and absence of the suppressors. These results suggested that in the mutant LPS backgrounds, *sfaA1* and *sfaB3* were able to restore OmpF to the outer membrane, possibly by correcting the assembly defect caused by the mutant LPS. Unfortunately, the λ phage cross-streak experiments did not provide any insight with respect to LamB assembly in the LPS mutant background.

In order to examine whether the presence of the suppressor allele, *sfaA1*, restored the assembly of OmpF and LamB in the Δ*rfa* strains, trimer assays using OmpF and LamB trimer-specific antibodies were performed. The results of the OmpF trimer assay are shown in Fig. 8. In the wild-type strain, the presence or absence of the suppressor allele had no effect on the trimerization of OmpF. However, in the LPS mutant strain, *sfaA1* was able to restore trimerization kinetics to the level observed in the wild-type *sfaA* strain, which produces complete LPS molecules. This *sfaA1* effect on OmpF assembly in the *rfa* deletion background was quantified by calculating the ratio of OmpF trimers to monomers (Fig. 9). The quantitative data clearly showed that the presence of the *sfaA1* suppressor allele alleviated the OmpF assembly defect observed in the LPS mutant strains.

The second suppressor allele, *sfaB3*, was also examined for its ability to restore OmpF to the outer membrane in Δ*rfa* strains. Trimer assays similar to those utilized for *sfaA1* were performed with trimer-specific antibodies. The results showed that in *rfa*$^+$ strains, the suppressor allele had no effect on OmpF trimerization. However, in Δ*rfa* strains, the suppressor allele, *sfaB3*, was able to restore OmpF trimerization kinetics to the wild-type level (data not shown).

Trimer assays using LamB trimer-specific antibodies were also performed, and the autoradiograph is shown in Fig. 10. As observed with OmpF, in the presence of wild-type LPS, the *sfaA1* allele had no effect on LamB trimerization. However, the presence of the suppressor allele restored the kinetics of LamB trimerization in Δ*rfa* strains. This effect of *sfaA1* was also quantified and is shown in Fig. 9. The results showed that in Δ*rfa* strains, the OmpF assembly mutant suppressor *sfaA1*

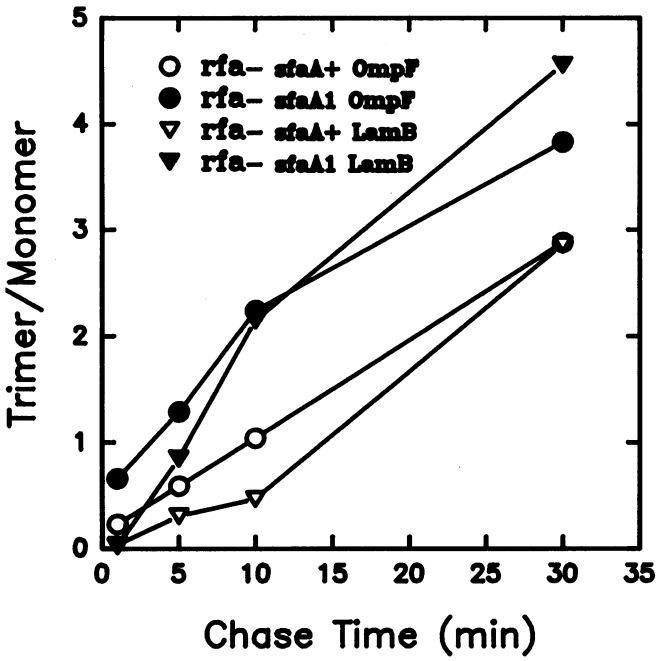

FIG. 9. Quantification of gels shown in Fig. 8 and 10. Ratios of trimer to monomer were plotted. Only the effects of *sfaA1* and *sfaA*$^+$ in Δ*rfa* strains are shown.

had the ability to restore LamB trimerization to the wild-type level. Since in LPS mutant backgrounds, LamB assembly was affected at both steps A and B (Fig. 6), it was of interest to see which of the two steps was corrected by *sfaA1*. We examined the fate of LamB monomers in the absence or presence of the suppressor allele in LPS deletion backgrounds. The results showed that *sfaA1* was unable to correct the assembly defect seen at step A (data not shown). Thus, *sfaA1* corrects the LamB assembly defect by acting at the step involving conversion of metastable trimers to stable trimers.

DISCUSSION

We have shown that LPS affects the trimerization kinetics of the outer membrane proteins OmpF and LamB. The effect of LPS on porin assembly is more evident for OmpF than for

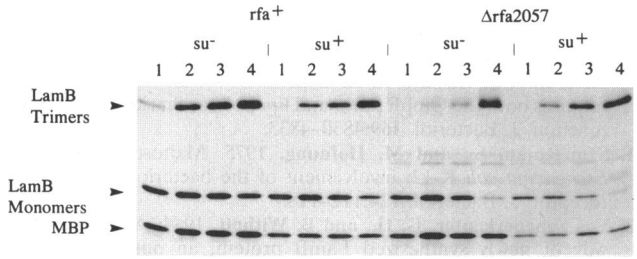

FIG. 10. LamB trimer assays of wild-type and mutant LPS strains in the presence and absence of the *sfaA* suppressor (su) allele. Growth and labeling conditions were similar to those described for Fig. 2. Chase samples were removed after 1, 5, 10, and 30 min (lanes 1 to 4, respectively). The samples were lysed, immunoprecipitated with trimer-specific LamB antibodies, and analyzed similarly to those in Fig. 2. Positions of the relevant protein bands are shown. MBP, maltose-binding protein.

LamB. This difference may be due to the unrelated protein structures, in addition to possible differences in assembly processes. In the Δ*rfa* strains, trimerization occurred at a reduced rate compared with that in the wild-type strain, thus indicating a role of wild-type LPS during the assembly of OmpF and LamB. The LPS effect on porin assembly may be during localization of porins to the outer membrane or it may simply facilitate the assembly of intermediates already localized in the outer membrane. The deep rough mutants used in this study lacked several *rfa* genes involved in the synthesis of the outer LPS core (*rfaQGBI*) and in phosphorylation of the heptose residues (*rfaP*) (10, 20). It has been shown that the loss of *rfaP* alone can result in a deep rough phenotype (24). Moreover, the deep rough phenotype and K20 resistance of the Δ*rfa* strains used in this study can be complemented by *rfaP*$^+$ plasmids (20). This suggests that the assembly defect seen in Δ*rfa* strains is due to the presence of unphosphorylated heptose residues and not due to the lack of outer core sugars. Previous studies from other laboratories have also provided evidence for the role of LPS in the assembly of OmpF (4, 9, 22). However, in these studies either a fatty acid synthesis inhibitor (4, 9) or genetically undefined LPS mutants (22) were used.

Previous in vitro work by Eisele and Rosenbusch (8) has shown the ability of OmpF to trimerize in the absence of LPS. However, in these in vitro studies it was not possible to establish the kinetic role of LPS in the assembly process as shown by the in vivo studies presented here. In separate studies carried out in Nikaido's laboratory, it was shown that the in vitro trimerization of OmpF required either full-length LPS or LPS containing at least the outer core (27, 28). Those results are consistent with the findings presented in this paper.

In the mutant LPS backgrounds, the rate of formation of OmpF stable trimers was considerably slower, which led to a transient buildup of metastable trimers. Thus, the interaction point during OmpF assembly appears to be at the step between metastable trimers and mature trimers. This observation is consistent with results obtained by Fourel et al. (9). Those authors used various OmpF monoclonal antibodies to show that the folding step from monomer to metastable trimer occurred independent of LPS, while the metastable-to-stable trimer conversion required fatty acid synthesis. Our data showed that during LamB assembly, LPS interacts not only at the metastable trimer-to-stable trimer step, but also at the step of conversion of monomers to metastable trimers. This resulted in the accumulation of both monomers and metastable trimers.

The site of interaction of LPS with assembling intermediates is not known. If this interaction occurs within the outer membrane, only the assembly intermediates that are localized in the outer membrane would be affected. In a previous study involving a mutant LamB, it was shown that LamB monomers can be localized to the outer membrane (16). In contrast, two separate studies have shown that newly synthesized OmpF monomers are first secreted into the periplasmic space prior to assembling into trimers (14, 26). Accordingly, the LamB monomer-to-metastable trimer step could be influenced by LPS, while this step may not be affected during OmpF assembly. Clearly, the determination of the LPS interaction site during porin assembly is extremely significant, and our current efforts are directed toward resolving this issue.

The most striking result obtained in this study was that *sfaA1* and *sfaB3* were able to restore assembly in LPS mutant backgrounds. The *ompF* assembly mutants described previously possessed structurally altered OmpF proteins that were unable to fold properly and thus could not assemble into stable

trimers (15). While the suppressors *sfaA1* and *sfaB3* restored assembly of these mutant OmpF proteins, they exerted no effect on the assembly of wild-type OmpF. However, when the *rfa* locus was partially deleted in order to produce defective LPS molecules, the resulting defect in wild-type OmpF assembly was corrected by *sfaA1* and *sfaB3*. In addition, these suppressor alleles were also able to restore LamB assembly in LPS mutant backgrounds. Furthermore, we have shown that *sfaA1* corrects the LamB assembly defect by acting at the step of metastable trimer-to-stable trimer conversion.

While it is not clear how the suppressors work, some explanations for their role in assembly can be provided. We have previously suggested that one way these suppressors might act is by permitting the assembly of certain structurally altered assembly intermediates (15). Genetic data suggested that the normal role of the wild-type *sfaA* and *sfaB* alleles is to prevent assembly of structurally altered proteins. Since the suppressor alleles of *sfaA* and *sfaB* restore assembly of both the mutant OmpF proteins and wild-type OmpF and LamB in mutant LPS backgrounds, it is tempting to propose that the lack of a proper interaction with LPS causes a structural alteration in the porin proteins during assembly. In this regard, the role of LPS could be considered to be similar to that of an assembly factor.

ACKNOWLEDGMENTS

We thank Leanne Misra for critical reading of the manuscript and Carl Schnaitman for providing LPS mutants.

This work was supported by NIH grant GM48167 to R.M.

REFERENCES

1. **Ames, G. F.-L., E. N. Spudich, and H. Nikaido.** 1974. Protein composition of the outer membrane of *Salmonella typhimurium*: effect of lipopolysaccharide mutations. J. Bacteriol. **117:**406–416.
2. **Baker, K., N. Mackman, and I. B. Holland.** 1987. Genetics and biochemistry of the assembly of proteins into the outer membrane of *E. coli*. Prog. Biophys. Mol. Biol. **49:**89–115.
3. **Benz, R.** 1988. Structure and function of porins from gram-negative bacteria. Annu. Rev. Microbiol. **42:**359–393.
4. **Bolla, J.-M., C. Lazdunski, and J.-M. Pages.** 1988. The assembly of the major outer membrane protein OmpF of *Escherichia coli* depends on lipid synthesis. EMBO J. **7:**3595–3599.
5. **Casadaban, M. J.** 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **141:**541–555.
6. **Clement, J.-M., and M. Hofnung.** 1981. Gene sequence of the λ receptor, an outer membrane protein of *E. coli* K-12. Cell **27:**507–514.
7. **Diedrich, D. L., M. A. Stein, and C. A. Schnaitman.** 1990. Associations of *Escherichia coli* K-12 OmpF trimers with rough and smooth lipopolysaccharides. J. Bacteriol. **172:**5307–5311.
8. **Eisele, J.-L., and J. P. Rosenbusch.** 1990. In vitro folding and oligomerization of a membrane protein. J. Biol. Chem. **265:**10217–10220.
9. **Fourel, D., S. Mizushima, and J.-M. Pages.** 1992. Dynamics of the exposure of epitopes on OmpF, an outer membrane protein of *Escherichia coli*. Eur. J. Biochem. **206:**109–114.
10. **Helander, I. M., M. Vaara, S. Sukupolvi, M. Rhen, S. Saarela, U. Zahringer, and P. H. Makela.** 1989. *rfaP* mutants of *Salmonella typhimurium*. Eur. J. Biochem. **185:**541–546.
11. **Kloser, A. W., and R. Misra.** Unpublished observations.
12. **Koplow, J., and H. Goldfine.** 1974. Alterations in the outer membrane of the cell envelope of heptose-deficient mutants of *Escherichia coli*. J. Bacteriol. **117:**527–543.
13. **Lugtenberg, B., J. Meijers, R. Peters, P. van der Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of the major outer membrane proteins of *Escherichia coli* K-12 into four bands. FEBS Lett. **58:**254–258.
14. **Metcalfe, M., and I. B. Holland.** 1980. Synthesis of a major outer membrane porin by *E. coli* spheroplasts. FEBS Lett. **7:**111–114.
15. **Misra, R.** 1993. OmpF assembly mutants of *Escherichia coli* K-12: isolation, characterization, and suppressor analysis. J. Bacteriol. **175:**5049–5056.
16. **Misra, R., A. Peterson, T. Ferenci, and T. Silhavy.** 1991. A genetic approach for analyzing the pathway of LamB assembly into the outer membrane of *Escherichia coli*. J. Biol. Chem. **266:**13592–13597.
17. **Muhlradt, P. F., and J. R. Golecki.** 1975. Asymmetrical distribution and artifactual reorientation of lipopolysaccharide in the outer membrane bilayer of *Salmonella typhimurium*. Eur. J. Biochem. **51:**343–352.
18. **Nakae, T.** 1976. Outer membrane of *Salmonella*. Isolation of protein complexes that produce transmembrane channels. J. Biol. Chem. **251:**2176–2178.
19. **Nikaido, H., and M. Vaara.** 1985. Molecular basis of bacterial outer membrane permeability. Microbiol. Rev. **49:**1–32.
20. **Parker, C. T., A. W. Kloser, C. A. Schnaitman, M. A. Stein, S. Gottesman, and B. W. Gibson.** 1992. Role of the *rfaG* and *rfaP* genes in determining the lipopolysaccharide core structure and cell surface properties of *Escherichia coli*. J. Bacteriol. **174:**2525–2538.
21. **Randall-Hazelbauer, L., and M. Schwartz.** 1973. Isolation of the bacteriophage lambda receptor from *Escherichia coli*. J. Bacteriol. **116:**1436–1446.
22. **Reid, G., I. Hindennach, and U. Henning.** 1990. Role of lipopolysaccharide in assembly of *Escherichia coli* outer membrane proteins OmpA, OmpC, and OmpF. J. Bacteriol. **172:**6048–6053.
23. **Reid, J., H. Fung, K. Gehring, P. E. Klebba, and H. Nikaido.** 1988. Targeting of porin to the outer membrane of *Escherichia coli*. Rate of trimer assembly and identification of a dimer intermediate. J. Biol. Chem. **263:**7753–7759.
24. **Schnaitman, C. A.** 1993. Personal communication.
25. **Schnaitman, C. A., and J. D. Klena.** 1993. Genetics of lipopolysaccharide biosynthesis in enteric bacteria. Microbiol. Rev. **57:**655–682.
26. **Sen, K., and H. Nikaido.** 1990. In vitro trimerization of OmpF porin secreted by spheroplasts of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**743–747.
27. **Sen, K., and H. Nikaido.** 1991. Lipopolysaccharide structure required for in vitro trimerization of *Escherichia coli* OmpF porin. J. Bacteriol. **173:**926–928.
28. **Sen, K., and H. Nikaido.** 1991. Trimerization of an in vitro synthesized OmpF porin of *Escherichia coli* outer membrane. J. Biol. Chem. **266:**11295–11300.
29. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Silverman, J. A., and S. A. Benson.** 1987. Bacteriophage K20 requires both the OmpF porin and lipopolysaccharide for receptor function. J. Bacteriol. **169:**4830–4833.
31. **Szmelcman, S., and M. Hofnung.** 1975. Maltose transport in *Escherichia coli* K-12: involvement of the bacteriophage lambda receptor. J. Bacteriol. **124:**112–118.
32. **Vos-Scheperkeuter, G. H., and B. Witholt.** 1984. Assembly pathway of newly synthesized LamB protein, an outer membrane protein of *Escherichia coli* K-12. J. Mol. Biol. **175:**511–528.