In Vitro Insertion and Assembly of Outer Membrane Protein PhoE of Escherichia coli K-12 into the Outer Membrane

ROLE OF TRITON X-100*

(Hans de Cock§†, Saskia van Blokland§, and Jan Tommassen‡§
From the Institute of Biomembranes and the §Department of Molecular Cell Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands)

The assembly of the in vitro synthesized outer membrane protein PhoE into purified outer membranes was investigated. The assembly appeared to be strongly stimulated by the presence of low amounts of Triton X-100 (optimal 0.08%, w/v). The role of Triton X-100 in the in vitro system was further examined. Pretreating outer membranes with Triton X-100 did not make the membranes competent for correct assembly, indicating that the detergent did not act on the membrane but at the protein level. PhoE became assembly-incompetent with a half-life of approximately 12 min and 90 s at 37 °C in the absence and presence, respectively, of 0.08% Triton X-100. Apparently, Triton X-100 induces an assembly-competent state in the PhoE protein with a very short half-life. Furthermore, the efficiency of correct assembly of PhoE was greatly reduced when outer membranes of deep rough lipopolysaccharide mutants were used, indicating an important role of lipopolysaccharides in the assembly of the porin.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Recipient of a fellowship from the Royal Netherlands Academy of Arts and Sciences. To whom correspondence should be addressed. Tel.: 31-30-2533184; Fax: 31-30-2513655.
‡ The abbreviations used are: OM, outer membrane(s); TX-100, Triton X-100; IM, inner membrane(s); LPS, lipopolysaccharide; mAb, monoclonal antibody; PAGE, polyacylamide gel electrophoresis.

The outer membrane (OM) of Escherichia coli K-12 contains three related proteins, OmpF, OmpC, and PhoE, that form general pores through which small hydrophilic solutes can pass (1, 2). The functional unit of these porins is a trimer. Intriguing questions are how these proteins reach their final destination in the cell and how they are assembled into trimers. We have described an in vitro system to study the assembly of PhoE protein (3). Using monoclonal antibodies that recognize conformational epitopes, it could be demonstrated that an in vitro synthesized quasi-mature PhoE protein could fold into a monomeric configuration, resembling a native subunit in the trimer. High concentrations of Triton X-100 (TX-100) (optimal 2%, w/v) could induce the formation of heat-stable trimers, resembling the in vivo formed native trimers, whereas the presence of purified OM drastically increased the kinetics of this process (3, 4). The OM components required for trimerization activity were not identified. However, interactions between outer membrane proteins and lipopolysaccharides (LPS) have been demonstrated (5) and suggested to be implicated in the assembly process (6–12). purified LPS did not induce assembly of PhoE trimers in this in vitro system (3), showing that LPS is either not involved or not sufficient for the assembly process. In addition, trimers of OmpF (13, 14) have been recently reconstituted in the absence of LPS. However, in this case detergents and/or phospholipids were used that might mimic the LPS activity.

In the present work, we attempted to extend the studies on the in vitro assembly of PhoE by developing a system to study the insertion of the protein into the OMs. Recently, it has been shown that low amounts of TX-100 (0.03%) can induce the insertion and assembly of the mature OmpF protein, secreted by spheroplasts (13), into purified OMs in vitro. Furthermore, OmpF protein, synthesized in an in vitro transcription-translation system, was assembled into trypsin-resistant trimers in the presence of OM (15), suggesting correct insertion of OmpF into OMs. In this latter system, the presence of TX-100 was not required for trimerization. In contrast, in vitro synthesized PhoE protein was not assembled into a trypsin-resistant configuration when incubated with OM in the absence of TX-100 (16). Here, we demonstrate that low amounts of TX-100 strongly stimulate the assembly of PhoE into purified OMs, and we investigated the role of the detergent and of LPS in this process in more detail.

MATERIALS AND METHODS

Bacterial Strains—S135 cell extracts were isolated from the E. coli K-12 strain MC4100 (17). Membranes were also isolated from this strain, as well as from E. coli U20 strains containing smooth LPS (18), galU mutant MC100 containing LPS of chemotype Rd (19), and CE1229, a derivative of MC4100, containing heptose-deficient LPS (20). The LPS content was determined by 3-deoxy-D-manno-octulosonic acid measurements (21) after precipitation of the membranes with acetone to remove the sucrose.

In Vitro Translations and Immunoprecipitations—Isolation of S135 cell extracts, preparation of the membranes, and the in vitro transcription and translation reactions were performed as described previously (22). Shortly thereafter, plasmid pJL370 (16) was used to direct the synthesis of a quasi-mature PhoE protein, containing, instead of the signal sequence, only a methionine and a serine at the N terminus of complete mature PhoE. Correctly folded proteins were immunoprecipitated with mAb PP1-1 (23) and protein A-Sepharose CL-4B. Immunocomplexes were dissociated by incubation in 200 mM glycine/HCl, pH 2.5, 30 min after initiation of translation, and the solution was for a short time mixed on a Vortex mixer and incubated for another 30 min at 37 °C. To study the association of PhoE to the membranes, the total mixture was centrifuged for 30 min.

1 The abbreviations used are: OM, outer membrane(s); TX-100, Triton X-100; IM, inner membrane(s); LPS, lipopolysaccharide; mAb, monoclonal antibody; PAGE, polyacylamide gel electrophoresis.

2 M. Struyve, M. Heutink, M. Kleerebezem, T. Van der Krift, H. de Cock, and J. Tommassen, submitted for publication.
at 40,000 rpm in a TLA 100 rotor (Beckman Instruments) at 15 °C. The pellet, containing the OMs, was resuspended in 20 μl of buffer L (50 mM triethanolamine acetate, pH 7.5, 250 mM sucrose, 1 mM dithiothreitol). To determine the amount of correctly assembled (i.e. protease-resistant) PhoE in the OM, 1 μl of trypsin (1400 μg/ml in 100 mM Tris-HCl, pH 8.0, 50 mM MgCl₂) was added, and the mixture was incubated for 30 min at 37 °C. Trypsin-resistant forms were detected and resuspended in buffer L. After 45 min of incubation at 37 °C, proteins were either immunoprecipitated with mAb PP1-1 or directly analyzed by SDS-PAGE.

SDS-Polyacrylamide Gel Electrophoresis and Quantifications—The LPS chemotype was analyzed by SDS-PAGE (18). Proteins were separated on SDS-polyacrylamide gels as described (26). Prior to electrophoresis, protein aliquots were incubated in sample buffer for 10 min at room temperature to detect folded monomers and at 56 °C to detect heat-stable trimers. Electrophoresis was performed at 4 °C in a temperature-controlled room at 20 mA in those cases where folded proteins had to be detected on the gels. Otherwise, gels were run at 30 mA at room temperature. Proteins in the gels were fixed and stained with 0.1% amido black/0.1% acetic acid. Gels were incubated for 30 min with Amplify (Amer sham Corp.) and dried at 80 °C. X-ray films were exposed at −80 °C for appropriate time periods to be within the linear exposure range. Quantifications were performed on an LKB 2222–010 Ultrasonic-XL laser densitometer. The amount of heat-stable trimers (56 °C) was calculated by using the formula: M_r [0°C] − M_r [56°C] × 100%, where M_r is the amount of PhoE monomers determined at the indicated temperature.

To determine the amount of radiolabeled protein synthesized in vitro, [35S]methionine-labeled proteins were separated by SDS-PAGE. Gels were dried after staining of the proteins as described above. To extract radiolabeled PhoE, gel pieces were incubated for approximately 18 h at room temperature in the dark with Lumasolve/water/Lipoluma as described by the manufacturer (Lumar L SC B.V.). The amount of radiolabeled proteins in the original sample was calculated after determining the amount of disintegrations/min in a scintillation counter (Beckman LS6000SE). Data were corrected for background radioactivity levels, which were determined with gel pieces of equal size from the same gel, and for incorporation of non-radioactive methionine, which was present in the S135 extract.

Treatment of OMs with Detergents—OMs were first isolated from a crude membrane fraction, containing both IMs and OMs, by centrifugation for exactly 5 min, not including the time required to start and stop the rotor, at 40,000 rpm in a Ti-50 rotor (Beckman Instruments) at 4 °C. This step provides specific separation of OMs from IMs (16). The pellet containing the OMs was dissolved in buffer L. Subsequently, 10 μl of OMs (6.65 nmol of LPS) were mixed with 65 μl of buffer L and 25 μl of a detergent solution in water. After incubation for 30 min at room temperature, OMs were reisolated by centrifugation in a TLA 100.2 rotor (30 min, 40,000 rpm at 15 °C) and resuspended in 100 μl of buffer L. Aliquots of pellet (OMs) and supernatant fractions (containing extracted material) were rapidly frozen in liquid nitrogen and stored at −80 °C.

RESULTS

TX-100 Stimulates Insertion and Assembly of PhoE into the Outer Membrane—In vitro synthesized PhoE is resistant to trypsin degradation when correctly assembled in the OM (27). Trypsin resistance can therefore be used as a criterion to establish whether PhoE is correctly assembled in vitro. To investigate whether low concentrations of TX-100 can induce the correct insertion and assembly of PhoE into OMs, quasi-mature PhoE was synthesized in vitro and incubated with OMs in the absence or presence of TX-100. Subsequently, OMs were pelleted and resuspended in buffer L. After 30 min of incubation at room temperature with trypsin, trimers were extracted from the membranes, and the folded proteins were immunoprecipitated with mAb PP1-1, which recognizes a conformational epitope, and analyzed by SDS-PAGE (Fig. 1). Indeed, trypsin-resistant trimers could be extracted from the OMs (Fig. 1). Optimal insertion was observed with 0.06% (lanes g and h) to 0.1% TX-100 (lanes i and j). Only very low amounts of protein were inserted into OMs in the absence of TX-100 (lanes a and b; visible only after long exposure of the film). In the absence of OMs, no protease-protected proteins were detected (lanes q and r). Some insertion was observed when IMs instead of OMs were used (lanes a and p; visible only after long exposure of the film), but this is most likely due to the presence of small amounts of OMs in this membrane fraction.

When the protein samples containing trypsin-resistant PhoE were not heated before electrophoresis (Fig. 1), several distinct forms of PhoE could be detected. Next to the expected forms, i.e. dimers (visible only after longer exposure times) and trimers, a monomeric form was detected, migrating slightly faster (M_r of 36,000) than the completely denatured PhoE. This form is not identical to the previously described folded monomer, which has an M_r of 31,000 (3). Since this 36-kDa species was immunoprecipitated with the conformation-dependent mAb, it could represent a pool of inserted folded monomers that may be an intermediate in the assembly process. Alternatively, it may arise by denaturation of intermediates (folded monomers, dimers, or unstable trimers) of the assembly process. The 36-kDa species was not always observed in different experiments.

Trypsin-resistant PhoE proteins were not extracted from the OMs after incubation for 30 min with 4 mM urea at 0 °C before centrifugation in buffer L, indicating that these proteins were integrated into and not peripherally associated with the OMs (data not shown). Analysis of the kinetics of the process indicated that the insertion of PhoE into the OM was completed within approximately 3 min at 37 °C (data not shown).

The Efficiency of Insertion into OMs Depends on the OM Concentration—Insertion of PhoE into OMs is expected to depend on the amount of available insertion sites in the OM. Therefore, insertion experiments were performed with various amounts of OMs isolated from strain U20. After trypsin treatment, OMs were pelleted, and both the OMs and supernatant fractions were analyzed (Fig. 2, A and B). With increasing amounts of OMs, a gradual increase in the amount of trypsin-resistant PhoE was observed to be inserted in the OM (Fig. 2, A and C) up to a certain maximum when apparently the...
amount of insertion-competent PhoE becomes limiting. Incubation of the samples at room temperature prior to electrophoresis revealed that trimers, as well as small amounts of folded monomers, were present (Fig. 2A). The trimers were resistant to denaturation in sample buffer at 56°C, comparable with the heat-stable trimers formed in vivo.

The relative efficiency of insertion of PhoE into OMs varied considerably between different experiments, and in some experiments, up to 50% of the total amount of protein synthesized was assembled into OMs. The variation in efficiency might result from the different amounts of PhoE protein synthesized in independent experiments. Quantification of the amount of protein synthesized in vitro in different experiments showed that it varied between 0.6 and 12 fmol of PhoE/50 µl of translation volume, which can explain the differences in the relative insertion efficiency at a constant amount of OMs. Therefore, one batch of in vitro synthesized protein was used in those cases where direct comparison of insertion efficiencies was required. Furthermore, the source of the OMs was of importance. Lower amounts of PhoE protein were assembled into OMs of strain MC4100 than assembled in those of strain U20 (Table I). In addition, only very low amounts of PhoE protein were assembled in an insertion-independent manner when incubated with OMs of the former strain. Therefore, OMs derived from strain U20 were used in further experiments. It is remarkable that relatively high amounts of outer membranes are required for insertion and assembly of very low amounts of PhoE protein. Overall, these data suggest either that the OMs used in these experiments contain limiting amounts of insertion sites or that a special, but limiting, subset of the isolated OMs is able to take up the PhoE protein.

Pretreatment of OMs with TX-100 does not make OMs competent for insertion—The role of TX-100 in the in vitro assembly of PhoE was further investigated. TX-100 might increase the competence of OMs in taking up outer membrane proteins, for example, by creating insertion sites. To test this possibility, OMs from strain U20 were pretreated with various amounts of TX-100, reisolated, and used in insertion assays with or without 0.08% TX-100 (Table II). To detect possible effects of the pretreatments on insertion of PhoE, OMs were used in assays supporting suboptimal insertion. Pretreatment of OMs with TX-100 was not sufficient to render them fully competent for insertion and assembly into trimers (Table II; +TX-100, PEL). In the absence of TX-100, some trypsin-resistant PhoE protein was inserted into the TX-100-pretreated OMs, but the majority of these were not assembled as heat-stable trimers but as folded monomers into the membrane. Addition of TX-100 (0.08%) during the incubation of PhoE with the membranes was again required to obtain correct insertion of PhoE as heat-stable trimers in the TX-100-pretreated OMs (Table II; +TX-100, PEL). Similar amounts of PhoE were inserted into OMs not pretreated with TX-100, and in both cases, approximately 50% of the inserted proteins were assembled into heat-stable trimers. These results demonstrate that pretreatment of OMs with TX-100 does not make them fully competent for PhoE assembly, suggesting that TX-100 works at the level of the PhoE protein, rather than on the membrane.

Pretreatment of OMs with TX-100 probably results in the formation of mixed micelles of the detergent with OM components or of OMs loaded up with TX-100. When no additional TX-100 is added, PhoE probably interacts with these TX-100/OM micelles resulting in the formation of trypsin-resistant folded monomers. Mixed micelles are probably also responsible for the presence of trypsin-resistant monomers in the superna-
centrifugation. Apparently, addition of 0.08% TX-100 to the
been released from the micelles and therefore not recovered by
(Table II; approximately 90 s in the presence of 0.08% TX-100 (Fig. 4
A)) to the assembly-competent state of PhoE at 37°C was reduced dras-
the amount of assembly-competent PhoE greatly decreased
PhoE to obtain an assembly-competent state. We noticed that
TX-100 might be required to modulate the conformation of
acts at the PhoE protein level rather than on the membranes.
results described in the previous section suggest that TX-100
with or without TX-100.
amounts of PhoE were correctly assembled in OMs pretreated
components required for efficient insertion, since similar
Interestingly, extraction of OMs with TX-100 did not remove
concomitant insertion, proceeds due to the presence of LPS or
PhoE protein into a trypsin-resistant configuration, without
TX-100 extraction. Thus, the assembly of large amounts of
insertion-independent assembly pathway (Table II) could
indeed be due to removal of LPS or other OM components by
TX-100 extraction. Thus, the assembly of large amounts of
PhoE protein into a trypsin-resistant configuration, without
concomitant insertion, proceeds due to the presence of LPS or
other OM components extracted from OMs by 0.08% TX-100.
Interestingly, extraction of OMs with TX-100 did not remove
components required for efficient insertion, since similar
amounts of PhoE were correctly assembled in OMs pretreated
with or without TX-100.
TX-100 Affects the Assembly-Competent State of PhoE—The
results described in the previous section suggest that TX-100
acts at the PhoE protein level rather than on the membranes.
TX-100 might be required to modulate the conformation of PhoE
to obtain an assembly-competent state. We noticed that
the amount of assembly-competent PhoE greatly decreased
when PhoE was preincubated with TX-100. The half-life of the
assembly-competent state of PhoE at 37°C was reduced dras-
tically from approximately 12 min in the absence (Fig. 4A) to
approximately 90 s in the presence of 0.08% TX-100 (Fig. 4B).
The half-life of the assembly-competent state in the presence of
0.08% TX-100 appeared to be temperature-dependent, since it
was substantially longer at 0 than at 37°C (data not shown).
These data suggest that TX-100 affects the folding of PhoE, since
folding is temperature-dependent.
Recently, we have demonstrated that removal of the C-ter-
phenoxy-D-octulosonic acid analysis of the fractions.

table II

| OM pretreatment | PEL | SUP | PEL | SUP |
|-----------------|-----|-----|-----|-----|
|                 | Tryp<sup>A</sup> | T | Tryp<sup>A</sup> | T |
| No TX-100       | 0.1 | —  | 3   | 63  |
| 0.08% TX-100    | 0.7 | 0.4 | 3   | 49  |
| 2% TX-100       | 25  | 4   | 2   | 65  |

Table I

Comparison of the assembly efficiency of PhoE with OMs of different strains
Experiments were performed as described in the legend of Fig. 2. The amount of trypsin-resistant PhoE protein present in the OMs pellet (P), the supernatant (S), and the total amount (T) are indicated as percent of the total amount of PhoE synthesized. Results of a representative experiment are shown in which OMs of MC4100 and of U20 were directly compared using a single translation mixture.

| LPS | MC4100 | U20 |
|-----|--------|-----|
| nmol/ml | P | S | T | P | S | T |
| 0.6  | 13  | 0.4 | 13.4 | 25.5 | 59 | 84.5 |
| 1.2  | 15  | 2.1 | 17.1 | 38.2 | 35 | 73.2 |

Figure 3. Removal of LPS from OMs pretreated with TX-100. OMs were treated with detergent as described under "Materials and Methods." The amount of LPS present in the supernatant fraction, obtained after resolation of the OMs by centrifugation, is indicated by percent of total amount present in the initial OMs and was determined by 3-deoxy-c-manno-octulosonic acid analysis of the fractions.

Figure 4. Effect of TX-100 on the half-life of the assembly-competent state of PhoE. Proteins synthesized in vitro were incubated in the absence (panel A) or presence (panel B) of 0.08% TX-100 at 37°C. At the indicated times, 25-μl samples were removed, mixed with OMs (U20; 0.665 nmol of LPS), and incubated for 30 min at 37°C. TX-100 (0.08%) was added together with the OMs when precubinations were performed in the absence of TX-100. The total amount of trypsin-resistant PhoE was expressed as percent of full-length PhoE synthesized. The proteins synthesized are quasi-mature wild-type PhoE, precubinated in the absence (+) or presence of 0.08% TX-100 (●) and precursor PhoE (□) and a quasi-mature mutant PhoE protein lacking the C-terminal phenylala-
nine (●), both precubinated in the presence of 0.08% TX-100.

of insertion of the protein into OMs in vivo (28) and in vitro
(this study). This decreased efficiency appears not to be due to a
greatly decreased half-life of the assembly-competent state that was, both in the absence (data not shown) and in the presence
(Fig. 4B) of TX-100, similar to that of wild-type PhoE. The signal sequence of PhoE interferes with the in vitro folding
of PhoE into a native-like structure (3). In addition, it has been
Triton X-100 Induces an Assembly-competent State in PhoE

![Figure 5](image)

**Fig. 5. Effect of LPS chemotype on the insertion process.** Insertion assays were performed as described in the legend of Fig. 1A with OMs of MC4100 (Ra LPS), MC1000 (Rd[linf;1] LPS), and CE1229 (Re LPS). After immunoprecipitation of the OM-associated trypsin-resistant PhoE proteins with the conformation-dependent mAb PP1-1, proteins were analyzed by SDS-PAGE and fluorography. The insertion efficiency (percent) is expressed relative to the amount of PhoE protein correctly inserted in OMs of MC4100 (Ra LPS), which was taken as 100%. Only the relevant part of the gel, on which proteins were loaded after heating for 10 min at 95 °C in sample buffer, is shown.

The data support the idea that the signal sequence is able to retard the folding of the precursor of maltose-binding protein into its native state (29). Therefore, it has been suggested that an important role of the signal sequence is to keep the precursors in a translocation-competent state. However, the presence of the signal sequence did not increase the half-life of the assembly-competent state of the PhoE protein in the presence of TX-100 but only reduced the efficiency of assembly of this protein (Fig. 4B).

**Mutations in LPS Core Region Decrease the Efficiency of the Assembly of PhoE into OMs—**

Recently, we have shown that high concentrations of TX-100 (0.5% or more) can induce the folding of PhoE into heat-stable trimers (4). Other non-ionic detergents could not substitute for TX-100 unless they contained, like TX-100, a phenyl ring. Folded monomers of PhoE were detected rapidly after the addition of the detergent, but trimers appeared only very slowly and not at the expense of the amount of folded monomers. Therefore, the folded monomer did not seem to be a precursor of the trimer, and the trimer appeared to be formed from a folding intermediate with a very short half-life. At the low detergent concentration used in the present study (0.06–0.08%), entirely different processes appear to have occurred. At these low detergent concentrations, heat-stable trypsin-resistant trimers can be formed, but the presence of OM components is essential. Furthermore, trimerization occurs via a folding intermediate with a very short half-life, and finally, other non-ionic detergents could substitute for TX-100.

The reduced efficiency of assembly of PhoE into OMs of LPS mutants might be explained by reduced amounts of insertion sites in these OMs. Freeze-fracture electron microscopy has revealed that the OM contains particles that probably consist of protein and LPS complexes, stabilized by divalent cations (33, 34). However, particles can also be formed by divalent cations and LPS together (34, 35). Interestingly, OMs of LPS mutants contain reduced amounts of particles (35). It was proposed that particles in the outer monolayer of the OM form hemimicelles that make complementary impressions in the inner monolayer (24). These impressions create a local increase in surface curvature at the periplasmic side of the OM. Further research will be required to determine whether the derived particles represent insertion sites.

Acknowledgment—We thank Tom Claassen for his contribution in the initial assembly assay.

REFERENCES

1. Lugtenberg, B., and van Alphen, L. (1993) Biochim. Biophys. Acta 737, 51–115
2. Nikaido, H., and Vaara, M. (1985) Mirobiol. Rev. 49, 1–32

...
12890

Triton X-100 Induces an Assembly-competent State in PhoE

3. de Cock, H., Hendriks, R., de Vrije, T., and Tommassen, J. (1990) J. Biol. Chem. 265, 4646–4651
4. Van Gelder, P., de Cock, H., and Tommassen, J. (1994) Eur. J. Biochem. 226, 783–787
5. Diedrich, D. L., Stein, M. A., and Schnaitman, C. A. (1990) J. Bacteriol. 172, 5307–5311
6. Freudl, R., Schwarz, H., Klose, M., Movva, N. R., and Henning, U. (1985) EMBO J. 4, 3593–3598
7. Bolla, J. M., Lazdunski, C., and Page’s, J. M. (1988) EMBO J. 7, 3595–3599
8. Page’s, J. M., and Bolla, J. M. (1988) Eur. J. Biochem. 176, 655–660
9. Diedrich, D. L., Stein, M. A., and Schnaitman, C. A. (1990) J. Bacteriol. 172, 5307–5311
10. Ried, J., Fung, H., Gehring, K., Klebb, P. E., and Nikaido, H. (1988) J. Mol. Biol. 263, 7753–7759
11. Laird, M. W., Kloster, A. W., and Misra, R. (1994) J. Bacteriol. 176, 2259–2264
12. Sen, K., and Nikaido, H. (1991) J. Bacteriol. 173, 926–928
13. Sen, K., and Nikaido, H. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 743–747
14. Eisele, J.-L., and Rosenbusch, J. P. (1990) J. Biol. Chem. 265, 10217–10220
15. Sen, K., and Nikaido, H. (1991) J. Biol. Chem. 266, 11295–11300
16. de Cock, H., Hekstra, D., and Tommassen, J. (1990) Biochimie (Paris) 72, 171–182
17. Casadaban, M. J. (1976) J. Mol. Biol. 104, 541–555
18. Van der Ley, P., de Graaff, P., and Tommassen, J. (1986) J. Bacteriol. 168, 449–451
19. Casadaban, M. J., and Cohen, S. N. (1980) J. Mol. Biol. 138, 179–207
20. de Cock, H., Meeldijk, J., Overduin, P., Verkleij, A., and Tommassen, J. (1989) Biochim. Biophys. Acta 985, 313–319
21. Van Alphen, L., Verkleij, A., Leunissen-Bijvelt, J., and Lugtenberg, B. (1985) Eur. J. Biochem. 147, 401–407
22. Van der Ley, P., Amesz, H., Tommassen, J., and Lugtenberg, B. (1985) Eur. J. Biochem. 147, 401–407
23. Van Alphen, L., Van Alphen, W., Verkleij, A., and Lugtenberg, B. (1979) Biochim. Biophys. Acta 566, 233–243
24. Bosch, D., Leunissen, J., Verbrakel, J., de Jong, M., Van Erp, H., and Tommassen, J. (1986) J. Mol. Biol. 189, 449–455
25. Lugtenberg, B., Meijers, J., Peters, R., Van der Hoeck, P., and Van Alphen, L. (1975) FEBS Lett. 58, 254–258
26. Tommassen, J., and Lugtenberg, B. (1964) J. Bacteriol. 117, 327–329
27. Struyve, M., Moons, M., and Tommassen, J. (1981) J. Mol. Biol. 147, 257–260
28. Park, S., Liu, G., Topping, T., Cover, W. H., and Randall, L. L. (1988) Science 239, 1033–1035
29. Tommassen, J., and Lugtenberg, B. (1981) J. Bacteriol. 147, 113–123
30. Arnes, G. F. L., Spudich, E. N., and Nikaido, H. (1974) J. Bacteriol. 117, 406–416
31. Zardeneta, G., and Horowitz, P. (1994) Anal. Biochem. 223, 1–6
32. Verkleij, A., Lugtenberg, E. J. F., and Ververgaert, P. H. J. T. (1976) Biochim. Biophys. Acta 426, 581–586
33. Van Alphen, L., Verkleij, A., Leunissen-Bijvelt, J., and Lugtenberg, B. (1978) J. Bacteriol. 134, 1089–1096
34. Verkleij, A., Van Alphen, L., Bijvelt, J., and Lugtenberg, B. (1977) Biochim. Biophys. Acta 466, 269–282
In Vitro Insertion and Assembly of Outer Membrane Protein PhoE of Escherichia coli K-12 into the Outer Membrane: ROLE OF TRITON X-100
Hans de Cock, Saskia van Blokland and Jan Tommassen

J. Biol. Chem. 1996, 271:12885-12890.
doi: 10.1074/jbc.271.22.12885

Access the most updated version of this article at http://www.jbc.org/content/271/22/12885

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 35 references, 14 of which can be accessed free at http://www.jbc.org/content/271/22/12885.full.html#ref-list-1