Pore-forming Activity of OmpA Protein of Escherichia coli*

Etsuko Sugawara† and Hiroshi Nikaido

From the Department of Molecular and Cell Biology, University of California, Berkeley, California 94720

Escherichia coli outer membrane protein OmpA was purified to homogeneity, as a monomer, from a K12 derivative deficient in both OmpF and OmpC porins. When proteoliposomes reconstituted from the purified OmpA, phospholipids, and lithium dodecyl sulfate were tested for permeability to small molecules by osmotic swelling, it was found that OmpA produced apparently nonspecific diffusion channels that allowed the penetration of various solutes. The pore-forming activity was destroyed by the heat denaturation of the OmpA protein, and the use of an OmpA-deficient mutant showed that the activity was not caused by copurifying contaminants. The size of the OmpA channel, estimated by comparison of diffusion rates of solutes of different sizes, was rather similar to that of E. coli OmpF and OmpC porins, i.e. about 1 nm in diameter. The rate of penetration of L-arabinose caused by a given amount of OmpA protein, however, was about a hundredfold lower than the rate produced by the same amount of E. coli OmpF porin. The addition of large amounts of lithium dodecyl sulfate to the reconstitution mixture increased the permeability through the OmpA channel, apparently by facilitating the correct insertion of OmpA into the bilayer.

This protein is multifunctional. In addition to its function as a phage receptor (Datta et al., 1977), it can serve as a mediator in F-factor-dependent conjugation (van Alphen et al., 1977; Schweizer and Henning, 1977), and it is known to function in stabilizing the shape of bacteria (Sonntag et al., 1978). There exists one report suggesting that OmpA functions in the transport of amino acids (Manning et al., 1977). OmpA mutant of Salmonella typhimurium, however, showed an unaltered permeability to cephaloridine (Nikaido et al., 1977). Thus, it is not yet clear whether OmpA functions as a nonspecific diffusion channel.

Unlike E. coli OmpF and OmpC porins, which exist as trimers even in the presence of SDS, there is no evidence that OmpA forms an SDS-resistant oligomer. However, the protein F from Pseudomonas aeruginosa (Yoshimura et al., 1983; Nikaido et al., 1991), which is purified as a monomer, shows significant pore-forming activity in reconstitution assays. These observations indicate that there are monomeric porins. Furthermore, there are some similarities in amino acid sequences between P. aeruginosa protein F and E. coli OmpA (Duchene et al., 1988).

Because of these observations, we examined the possibility that E. coli OmpA may also show a pore-forming activity. The results indicate that indeed the OmpA protein produces a diffusion channel allowing a slow penetration of small solutes.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Their Cultivation—E. coli HN705 [K12A(lac-proAB) lacF lacZAM1, lacF lacZAM15], which is deficient in both of the classical E. coli porins OmpF and OmpC, was constructed by S. Oya in this laboratory as follows. First, a deletion mutation in ompC was transduced, with phage P1, together with a nearby Tn10 insertion zei-298:Tn10, from strain CS1253 (Schneck and McDonald, 1984) into strain JM101 (Messing et al., 1981). To one of the transductants confirmed to be deficient in OmpC protein by SDS-PAGE of the cell envelope fraction, ompF::Tn5 was further transduced in by P1 from strain M450 (Andrews and Sherr, 1986). The strain was kept frozen at –70 °C in glycerol; otherwise, the population tended to be replaced by suppressor mutants that can express alternative porins, as was previously found with other porin-deficient strains (Bavoil et al., 1977).

An OmpA-deficient spontaneous mutant of HN705, HN742, was obtained by selecting for resistance to phage Ksh1 (Manning et al., 1976). The mutant was also stored frozen at −70 °C. Cells were grown in L broth at 37 °C, with aeration by rotary shaking.

Preparation of OmpA Protein—A part of the outer membrane fraction obtained from 1 liter of exponentially growing culture as described previously (Nikaido et al., 1983), was suspended in 0.3% LDS containing 5 mM EDTA and 10 mM Tris-C1, pH 7.5, for 30 min at 0 °C. After centrifugation at 100,000 × g for 45 min, the supernatant was loaded onto a column of Sephacryl S-300 (1.5 × 90 cm) equilibrated with 0.1% LDS, 0.4 mM
LiCl, 10 mM Tris-Cl, pH 7.5, and the proteins were eluted with the same buffer. Fractions (1.5 ml) were analyzed by SDS-PAGE. OmpA-containing fractions were combined and concentrated by dialysis against Ficol 400. Salts were removed by passing samples through minicolumns of Sephadex G-25 (bed volume, 5 ml), which was equilibrated with 0.1% LDS, 10 mM Tris-Cl, pH 7.5. The OmpA preparation did not contain any major contaminant, as judged by the scanning of the Coomassie Blue-stained SDS-PAGE (see Fig. 1).

For denaturation of OmpA, the sample was heated at 100 °C for 1 h in the presence of 2% SDS. Gel filtration on a Sephacryl S-300 column was then performed to compare the difference in elution positions between the native and the denatured OmpA.

Liposome Swelling Assay—This was carried out essentially as described earlier (Nikaido et al., 1991), by using a mixture of acetone-washed egg phosphatidylcholine and dicetylphosphate as the phospholipid. However, in most experiments, we added 2 μmol of LDS to the reconstitution mixture containing 3.1 μmol of phosphatidylcholine in order to facilitate the correct insertion of OmpA into the phospholipid bilayer (see “Results”).

The extent of incorporation of the added OmpA into the liposome with or without LDS addition was examined by sucrose density gradient flotation centrifugation (Yoshimura et al., 1983). After fractionation, OmpA was detected by protein assay. Protein assay was also performed on the corresponding fractions from a control gradient containing liposomes without OmpA in order to correct for the interference by sucrose and possibly other components in the gradient.

Other Methods—The amount of LDS bound to the protein was determined by subjecting the proteins to gel filtration in the presence of LDS just above its critical micellar concentration; we used 0.1% LDS in 10 mM Tris-Cl, pH 7.5. The amount of LDS bound to the proteins was measured by the binding of methylene blue (Hayashi, 1975). SDS-PAGE was carried out as described by Lugtenberg et al. (1975). The samples for SDS-PAGE were heated for 5 min at 50 and 95 °C in the sample buffer. Protein was determined by the method of Lowry et al. (1951), with bovine serum albumin as the standard.

RESULTS

Purification of OmpA—To study whether OmpA protein has a pore-forming activity, we purified OmpA from the outer membrane of E. coli HN705, which is deficient in both of the conventional E. coli porins OmpF and OmpC, by gel filtration in the presence of LDS. Since OmpA becomes inactivated easily during purification, we devised a simple and rapid purification procedure to avoid its inactivation. Extraction in 0.3% LDS containing 5 mM EDTA and 10 mM Tris-Cl, pH 7.5, removed most of non-OmpA proteins from the outer membrane, and subsequent extraction in 2% LDS in the same buffer produced a preparation enriched in OmpA (Fig. 1). Then gel filtration was carried out on a column of Sephacryl S-300 in the presence of 0.1% LDS containing 0.4 M LiCl and 10 mM Tris-Cl, pH 7.5. Here, a high concentration of salt was necessary, since OmpA was eluted as large aggregates if gel filtration was done without 0.4 M LiCl. OmpA was eluted as a sharp peak. For use in the swelling assay, we needed to remove excess salt from the OmpA preparation. When the preparation containing OmpA was dialyzed against 10 mM Tris-Cl buffer, pH 7.5, 0.1% LDS overnight at 4 °C, OmpA was partly converted to the denatured form, corresponding to a “heat-modified” form, with an apparent molecular weight of 33,000 (Nakamura and Mizushima, 1976). These preparations had often lost activity significantly. A previous study in our laboratory also found a similar phenomenon with P. aeruginosa protein F (Yoshimura et al., 1983), which was unstable in a low ionic strength buffer and was converted into an inactive form during dialysis. Because of this instability, desalting of OmpA fractions was carried out using minicolumns of Sephadex G-25 equilibrated with 0.1% LDS, 10 mM Tris-Cl, pH 7.5, and this resulted in the reproducible preparation of OmpA, which had a full pore-forming activity and did not contain any heat-modified forms on SDS-PAGE (not shown).

In order to assess the aggregation state of OmpA prepared in this manner, two experiments were carried out. First, the OmpA preparation was completely denatured by heating at 100 °C for 1 h in 2% SDS. If OmpA originally existed as oligomers, denaturation will also result in the dissociation of the oligomer into a monomer. The OmpA is therefore expected to be eluted at a much later position. As shown in Fig. 2, however, this did not occur. In fact, the denatured OmpA was eluted slightly earlier than the unheated form, probably because the denatured, extended shape of the heated protein produces more friction during the gel filtration process. Second, the position of elution of denatured OmpA was compared with those of molecular weight standards, including rabbit muscle aldolase, bovine serum albumin, ovalbumin, and soybean trypsin inhibitor. These standards were eluted in the same buffer not containing LDS, because these proteins will be fully denatured in the presence of LDS (Fig. 2). The position of elution of OmpA was similar to that of bovine serum albumin (M, 67,000). This was significantly earlier than the position expected for the monomer of the OmpA protein. However, we found that an almost equal amount of

![Fig. 1. Purification of E. coli OmpA. SDS-PAGE patterns of some fractions are shown. Lane 1, molecular weight standards (see below); lane 2, crude outer membrane; lane 3, 0.3% LDS extract of the outer membrane; lane 4, extract obtained by the subsequent treatment with 2% LDS; lane 5, OmpA purified by gel filtration. Each sample was heated in sample buffer at 50 °C (left) or at 100 °C (right) for 5 min. Molecular weight standards were, from the top, phosphorylase b (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), and soybean trypsin inhibitor (21,500).](image1)

![Fig. 2. Elution profiles of undenatured and denatured OmpA. The samples, undenatured (○) or denatured (●) by heating at 100 °C for 1 h in the presence of 2% SDS, were applied to a column (1.5 × 90 cm) of Sephacryl S-300 equilibrated with 0.1% LDS, 0.4 M LiCl, 10 mM Tris-Cl, pH 7.5, and were eluted with the same buffer. The standard proteins, including rabbit muscle aldolase (158,000), bovine serum albumin (66,900), ovalbumin (45,000), and soybean trypsin inhibitor (21,500), were fractionated by elution with the same buffer, but not containing LDS.](image2)
LDS (0.95 ± 0.14 g/g of protein) was bound to OmpA under these conditions. This suggests that the apparent molecular weight of OmpA in the presence of LDS should be about 70,000, not far away from that of bovine serum albumin. Both of these approaches thus supported the conclusion that E. coli OmpA purified by us was monomeric.

**Pore-forming Activity of E. coli OmpA**—The pore-forming activity of purified OmpA from E. coli was determined by reconstitution into proteoliposomes and by the osmotic swelling of these vesicles detected by following the optical density of the suspension. As seen in Fig. 3, the permeability of liposomes toward L-arabinose was proportional to the amount of OmpA added. If the OmpA produced pores by first assembling into larger structures, such as dimers or trimers, a concave upward curve could have been expected.

We noticed that addition of LDS at the time of reconstitution increased the rate of change of optical density of OmpA-containing proteoliposomes (Fig. 3). The effect of LDS on the apparent activity was dependent upon the amount added, reaching the maximum of about 5-fold stimulation at a ratio of 0.7 mol of LDS/mol of phospholipid (Fig. 4). Because of its negative charge, LDS may widen the distances between the successive layers of multiple layered proteoliposomes and increase the optical density changes caused by osmotic swelling. However, addition of LDS to the reconstitution mixture containing OmpF porin did not cause any increase of the rate of change of optical density (Fig. 4), and therefore, LDS appears to enhance truly the permeability of OmpA-containing proteoliposomes. Some detergents are known to facilitate the insertion of some integral membrane proteins into the phospholipid vesicles (Eytan et al., 1976). We therefore examined the extent of incorporation of OmpA into the proteoliposome with or without LDS by sucrose gradient flotation centrifugation. Ninety-eight percent of OmpA added was floated to the top fractions that contained liposomes, regardless of whether the reconstitution was with or without LDS addition. Among other detergents tested, including octyl β-D-glucopyranoside, dodecyl β-D-maltoside, zwittergent 3-14, Triton X-100, and Tween 80, only Triton X-100 and Tween 80 increased slightly, by 25%, the rate of optical density change of the OmpA-containing proteoliposomes upon their dilution into an iso-osmotic solution of L-arabinose.

It is known that LPS, especially its lipid part (lipid A), is required for phage receptor activity of OmpA (Schweizer et al., 1978). LDS from E. coli, however, did not affect noticeably a pore-forming activity when 5 nmol of LDS, about 5-fold molar excess over the OmpA protein, was added to the reconstitution mixture.

The permeability of OmpA-containing liposomes toward L-arabinose was much less than what was produced by the incorporation of an equivalent amount of E. coli OmpF porin, by a factor of about 100. Although the penetration of solutes into OmpA-containing proteoliposomes occurred so slowly, it appeared to occur through real protein channels rather than nonspecifically through protein-lipid interfaces. Thus, denaturation of OmpA, either by heating for 1 h at 100 °C in 2% SDS or by treatment for 5 min at room temperature in 0.1 N HCl, followed by neutralization with NaOH, resulted in a loss of more than 90% of pore-forming activity, as determined by the swelling of proteoliposomes in L-arabinose. Furthermore, no significant swelling was observed with proteoliposomes reconstituted with up to 60 μg of bovine serum albumin.

We estimated the size of OmpA channel by measuring the dependence of diffusion rates on the sizes of solutes (Fig. 5). The data showed that the size was very similar to that of the

**FIG. 3.** Swelling rate of liposomes reconstituted with *E. coli* OmpA. Different amounts of OmpA (0–60 μg) were reconstituted with 3.1 μmol of egg phosphatidylcholine and 0.2 μmol of dicetyl phosphate in the presence (●) or absence (○) of 2 μmol of LDS. Then, 13 μl of the proteoliposome suspensions were diluted into 0.6 ml of iso-osmotic L-arabinose, and the initial rates of OD50 decrease were measured. Each point is an average of five data, and the standard error was, on average, less than 10%.

**FIG. 4.** Effect of the amount of LDS added during reconstitution on the apparent pore-forming activities of OmpA and OmpF. OmpA (20 μg of protein) (●) and OmpF (0.4 μg of protein) (○) were reconstituted with 3.1 μmol of egg phosphatidylcholine and 0.2 μmol of dicetyl phosphate in the presence of various amounts of LDS. Then, 13 μl of the proteoliposome suspensions were diluted into 0.6 ml of iso-osmotic L-arabinose, and the initial rates of OD50 decrease were measured. Control liposomes (■), not containing any porin, were treated identically.

**FIG. 5.** Diffusion rates of solutes of various sizes through the *E. coli* OmpA channel. *E. coli* OmpA (20 μg of protein) was reconstituted into the phospholipid membrane as described under "Experimental Procedures." They were diluted in iso-osmotic solutions of L-arabinose (M, 150), d-glucose (M, 180), N-acetyl-D-glucosamine (M, 221), and sucrose (M, 342), and the initial rate of swelling of the liposomes was determined (●). The data for *E. coli* OmpF porin (A) and *P. aeruginosa* protein F (●) are from Nikaido and Rosenberg (1983) and from Nikaido et al. (1991).
OmpF channel and was much narrower than that of the *P. aeruginosa* protein F.

Because of the report suggesting that OmpA functions as an amino acid channel (Manning et al., 1977), we tested the diffusion of several amino acids and peptides. There was no sign, however, that OmpA specifically favored the diffusion of these solutes. For example l-glutamine, with a molecular weight similar to L-arabinose, diffused at 96% of the rate of L-arabinose.

**Pore-forming Activity of the OmpA-deficient Mutant**—Because OmpA allowed only a slow diffusion of solutes as described above, it was necessary, in its preparation, to minimize the contamination of classical OmpF and OmpC porins, which have nearly a 100-fold higher pore-forming activity. We therefore used the OmpF- and OmpC-deficient mutant strain, HN705, as starting material for purification of OmpA. With this strain grown in L broth, OmpA makes up about 40% of the total protein of the outer membrane, based on the scanning of the Coomassie Blue-stained SDS-PAGE slabs. Based on the relative abundance and the specific pore-forming activity, the presence of OmpA can explain most of the pore-forming activity of the outer membrane of this mutant. However, the low specific activity of OmpA left one alternative interpretation of our data open. If our OmpA preparation was contaminated by 1% of a protein with a pore-forming activity comparable with conventional porins, we could have obtained the results so far described. To address this point more directly, we used HN742, an OmpA-deficient mutant of HN705. In a control experiment, 2% LDS extract of the outer membrane from HN705 strain was fractionated on a column of Sephadryl S-300, and pooled fractions were assayed for pore-forming activity. As expected, about 60% of total activity was observed in pool II, which was enriched in OmpA (Fig. 6). In contrast, when the extract from an OmpA-deficient strain HN742 was fractionated, the peak of OmpA was indeed missing on the elution profile. Furthermore, the pore-forming activity of pool II was essentially absent (Fig. 6), indicating that the pore-forming activity of pool II (and also pool I) was indeed due to OmpA, not to invisible minor contaminant(s) in the OmpA fraction.

The mutant outer membrane, however, still retained about 30% of the permeability toward arabinose in comparison with the parent strain, even though all of the known porins were lacking. It is not yet clear which proteins are involved in the residual nonspecific diffusion process.

**DISCUSSION**

In this paper, we showed that the OmpA protein of *E. coli* produces diffusion channels. This observation is consistent with the pore-forming activity observed in a homologous protein, OprF or protein F, from *P. aeruginosa* (Yoshimura et al., 1983; Nikaido et al., 1991). The OprF and OmpA proteins also share some other phenotypic properties. Both have important structural roles in stabilizing the structure of the outer membrane (Sonntag et al., 1978; Gotoh et al., 1989; Woodruff and Hancock, 1989), and neither seems to exist as tightly associated trimers, being easily isolated as functional monomers in the presence of SDS. Furthermore, the rates of solute diffusion through both of these channels are about 2 orders of magnitude slower than through the OmpF or OmpC porins (Nikaido et al., 1991; this study). In terms of pore size, however, the *P. aeruginosa* OprF produced a large channel with an estimated diameter of about 2 nm (Nikaido et al., 1991), whereas the OmpA channel was similar to those of OmpF and OmpC porins, i.e. close to about 1 nm in diameter.

Because of this very slow penetration of solutes through the proteoliposome membranes containing the OmpA protein, we made some efforts to show that this permeation is not through a nonspecific interface between proteins and lipids or through the interior of denatured proteins. We could show that denaturation of OmpA essentially abolished the solute diffusion process and that nonporin proteins such as bovine serum albumin could not facilitate the solute diffusion across the membrane; we are thus convinced that the channel formation is an intrinsic function of the native OmpA protein. Furthermore, the experiment using the OmpA-deficient mutant HN742 (Fig. 6) showed conclusively that the channel formation was caused by OmpA, not by minor contaminating proteins that copurified with OmpA.

On the basis of these results, we can now recognize two major classes of porins, here defined as proteins producing nonspecific diffusion channels, in enteric bacteria and pseudomonads. One class contains the classical porins OmpF and OmpC, which exist as tightly associated trimers and produce channels of very high permeability. The second class is composed of *E. coli* OmpA protein and *P. aeruginosa* protein F. Neither of these latter proteins appears to exist as tightly associated oligomers. When reconstituted into liposome membranes, both proteins confer only low permeability, allowing only slow penetration of small solutes.

The low permeability produced by the OmpA protein explains the previous failure to detect porin activity in this protein. Thus, Nakae (1976) did detect some pore-forming activity in SDS extracts of the outer membrane, enriched in OmpA, but because he used a wild type strain producing porins that are about a hundredfold more effective in permeabilizing the liposomes, he could not rule out the possibility that this was caused by the contamination by OmpF and other trimeric porins. In cells deficient in OmpA alone, Nikaido et al. (1977) did not detect any decrease in permeability toward cephalosporins, but this is as expected because perhaps 99% of the penetration across the outer membrane would occur through trimeric porins and the presence or absence of OmpA would not produce detectable differences in permeability.
It was reported that OmpA-deficient strains are very defective in the uptake of amino acids (Manning et al., 1977). We have tested the diffusion of a few amino acids through the OmpA channel because of this report but could not find any evidence that the OmpA channel specifically favors the diffusion of amino acids or peptides (see “Results”). Although the diffusion of these solutes should ideally be examined at lower concentrations (Trias et al., 1989), the defective transport of amino acids in mutants deficient in OmpF (Bavoil et al., 1977), theoretical considerations on the permeability of E. coli outer membrane (Nikaido and Vaara, 1985), and the behavior of the OmpF/OmpC protein and OmpA protein in reconstituted systems do not favor the idea that OmpA is an amino acid-specific channel. Since Manning et al. (1977) measured the accumulation of amino acids into the cytoplasm, rather than their diffusion across the outer membrane, their results could have been affected by the poor viability of some ompA mutants under certain conditions.

An unexpected finding in this study was that the addition of LDS to the reconstitution mixture enhanced greatly the rate of change in optical density of OmpA-containing proteoliposomes as a control showed that most of this effect was a reflection of increased permeability of the OmpA-containing bilayer, and we suspect that LDS helped to bring about the correct insertion of OmpA proteins into the bilayer. In some ways, these results are not surprising. In contrast to OmpF/OmpC proteins that assume a very rigid, stable structure thanks to their trimeric aggregation, our OmpA preparation is monomeric and is thus expected to be affected much more by the nature of the surrounding lipids. Furthermore, our reconstitution is done by using phosphatidylcholine, which does not exist in E. coli. Thus, a LDS/phosphatidylcholine mixture presumably produces a bilayer that is more acceptable for the OmpA protein. Possibly LDS, as an acidic amphiphile, mimics LPS in the native outer membrane. If this is correct, bilayers containing large amounts of LPS should be even better, but because such bilayers produce very high turbidity, we were able to add only a small amount of LPS at the time of reconstitution, and this did not produce much difference in permeability.

Finally, we should consider the possible tertiary structure of OmpA in relation to the formation of the channel. Recently, the structure of one of the trimeric porins producing very high permeability, the Rhodobacter capsulatus porin, was solved by x-ray crystallography (Weiss et al., 1977). A monomer subunit was shown to cross the membrane 16 times as antiparallel β-strands. In contrast, OmpA is generally thought to cross the membrane only eight times as β-strands (Klose et al., 1988), and in this study, there was no indication that OmpA had to oligomerize in order to produce channels (see “Results”). Since the diameter of the OmpA channel estimated by liposome swelling assay (1.0 nm) is only a little narrower than the estimate for R. capsulatus porin (1.4 nm; Flammann and Weckesser 1984), at first it seems difficult to imagine that only eight β-strands could produce a channel of such width. However, with R. capsulatus porin, the narrow channel diameter is essentially determined by a portion of the protein folding into the channel, creating a structure called an “eyelet.” When this is taken into account, it does not appear impossible to create a channel of 0.8–1 nm in diameter from a β-barrel with eight strands. The circumference of such a barrel would be about 3.6 nm, based on the 0.45-nm distance between adjacent β-strands; this will produce a barrel with the backbone to backbone diameter of 1.15 nm. Since the amino acid residues predicted to face the channel interior in the model (Klose et al., 1988) tend to have unusually short side chains (11 residues out of 38 are glycine residues), perhaps an eight-strand barrel, without the eyelet structure, is capable of producing a diffusion channel of the width observed in this study.

The slow penetration rate of the solutes through the OmpA or OprF channel does not yet have a definite explanation in molecular terms. It is possible that the solutes have to push aside the flexible side chains of amino acid residues extending into the channel interior, and that this slows down the penetration process. On the other hand, only a fraction of the protein population might be capable of producing the channel, and the low value of the permeability coefficient may be only apparent, as has been proposed for the P. aeruginosa OprF channel (Nikaido and Hancock, 1986). More studies are needed to reach a decision between these alternative possibilities.

Acknowledgments—We thank Dr. Satoshi Oya for constructing strain HN742 and Dr. Ulf Henning for the gift of phage K3h1.

REFERENCES

Andrews, J. C., and Short, S. A. (1985) J. Bacteriol. 161, 484–492

Bavoil, P., Nikaido, H., and von Meyenburg, K. (1977) Mol. Gen. Genet. 158, 23–33

Chen, R., Schmidmayr, W., Kramer, C., Chen-Schneisser, U., and Henning, E. (1980) Proc. Natl. Acad. Sci. USA 77, 4592–4596

Datta, D. B., Arden, B., and Henning, U. (1977) J. Bacteriol. 131, 821–829

Duchene, M., Schweiner, A., Lottspeich, F., Krauss, G., Mazet, M., Vogel, K., von Specht, B.-U., and Domlely, H. (1988) J. Bacteriol. 170, 155–162

Byant, G., Matheson, M. J., and Backer, E. (1976) J. Biol. Chem. 251, 8681–8687

Flammann, H. T., and Weckesser, J. (1984) J. Bacteriol. 159, 410–412

Gotolh, N., Wakele, H., Yoshinaka, R., Nakae, T., and Nishimo, T. (1989) J. Bacteriol. 171, 983–990

Hayashi, K. (1976) Anal. Biochem. 70, 503–506

Klose, M., Maclntyre, S., Schwartz, H., and Henning, U. (1988) J. Biol. Chem. 263, 13297–13302

Lover, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–276

Lugtenberg, B., Meijers, J., Peters, R., van der Hoek, R., and van Alphen, L. (1975) FEBS Lett. 58, 254–259

Manning, P. A., Pupsers, A., and Reeves, P. (1976) J. Bacteriol. 127, 1081–1084

Manning, P. A., Pugsley, A. P., and Reeves, P. (1977) J. Mol. Biol. 116, 285–300

Messing, J., Cren, R., and Seeburg, P. H. (1981) Nucleic Acids Res. 9, 309–321

Nakae, T. (1976) J. Biol. Chem. 251, 2176–2179

Nakamura, K., and Mizushima, S. (1976) J. Bacteriol. 128, 1411–1422

Nikaido, H., and Hancock, R. E. W. (1986) in The Bacteria (Sokatch, J. R., ed.), Vol. X, pp. 145–193, Academic Press, Orlando, FL

Nikaido, H., and Rosenberg, E. G. (1983) J. Bacteriol. 153, 241–252

Nikaido, H., and Vaara, M. (1988) Microbiol. Rev. 49, 1–32

Nikaido, H., Song, S. A., Shaltiel, L., and Nurminen, M. (1977) Biochemistry 16, 324–330

Nikaido, H., Rosenberg, E. Y., and Foulds, J. (1983) J. Bacteriol. 153, 232–240

Nikaido, H., Nikaido, K., and Harayama, S. (1991) J. Biol. Chem. 266, 770–779

Schneitz, A. C., and McDonald, G. A. (1984) J. Bacteriol. 155, 556–563

Schweizer, M., and Henning, U. (1977) J. Bacteriol. 129, 1651–1652

Schweizer, M., Lindenach, I., Gaten, W., and Henning, U. (1978) Eur. J. Biochem. 82, 211–217

Sonntag, I., Schwartz, H., Hirota, Y., and Henning, U. (1978) J. Bacteriol. 136, 259–265

Trias, J., Dufrene, J., Levesque, R. C., and Nikaido, H. (1989) Anton. Microb. Agents Chemother. 34, 1201–1206

van Alphen, L., Mavekes, L., and Lugtenberg, B. (1977) FEBS Lett. 75, 285–289

Weber, M. S., Kreusch, A., Schilz, E., Nestel, U., Welte, W., Weckesser, J., and Schulz, E. C. (1991) FEBS Lett. 280, 379–382

Wosten, W. A., and Hancock, R. E. W. (1989) J. Bacteriol. 171, 3204–3209

Yoshimura, F., Zalman, L. S., and Nikaido, H. (1985) J. Biol. Chem. 258, 2308–2314