

Polyethyleneimine is an effective permeabilizer of Gram-negative bacteria

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The effect of the polycation polyethyleneimine (PEI) on the permeability properties of the Gram-negative bacterial outer membrane was investigated using *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* as target organisms. At concentrations of less than 20 µg ml⁻¹, PEI increased the bacterial uptake of 1-*N*-phenylnaphthylamine, which is a hydrophobic probe whose quantum yield is greatly increased in a lipid environment, indicating increased hydrophobic permeation of the outer membrane by PEI. The effect of PEI was comparable to that brought about by the well-known permeabilizer EDTA. Permeabilization by PEI was retarded but not completely inhibited by millimolar concentrations of MgCl₂. PEI also increased the susceptibility of the test species to the hydrophobic antibiotics clindamycin, erythromycin, fucidin, novobiocin and rifampicin, without being directly bactericidal. PEI sensitized the bacteria to the lytic action of the detergent SDS in assays where the bacteria were pretreated with PEI. In assays where PEI and SDS were simultaneously present, no sensitization was observed, indicating that PEI and SDS were inactivating each other. In addition, a sensitizing effect to the nonionic detergent Triton X-100 was observed for *P. aeruginosa*. In conclusion, PEI was shown to be a potent permeabilizer of the outer membrane of Gram-negative bacteria.

Keywords: polyethyleneimine, Gram-negative bacteria, outer membrane, lipopolysaccharide

INTRODUCTION

Polyethyleneimine (PEI) is a weakly basic aliphatic polymer which is polycationic due to the presence of primary, secondary and tertiary amino groups. PEIs are available in different molecular masses and forms, and they are widely utilized to flocculate cellular contaminants such as nucleic acids, lipids and debris from cellular homogenates to facilitate purification of soluble proteins (Cordes *et al.*, 1990; Kirk & Cowan, 1995; Milburn *et al.*, 1990; Salt *et al.*, 1995). Enzymic reactions in bioprocesses constitute another field where PEI is used, as an immobilizing agent for biocatalysts (Bahulekar *et al.*, 1991), as a soluble carrier of enzymes (Cong *et al.*, 1995), or in the formation of macrocyclic metal complexes mimicking metalloenzymes (Suh *et al.*, 1991). A survey of patent data reveals that PEI is a

common ingredient in a variety of formulations ranging from washing agents to packaging materials, and is also involved in microbicidal compositions. There are few data, however, concerning the microbicidal activity of PEI and its interaction with bacteria.

Certain polycationic substances such as polymyxin and its derivatives, polylysines and protamine are known to make the Gram-negative bacterial outer membrane (OM) permeable to solutes that normally are unable to penetrate it (for a review see Vaara, 1992). The OM is present in all Gram-negative bacteria, and due to the presence of the unique macromolecule LPS (for a review see Helander *et al.*, 1996) in the outer leaflet of the membrane, it forms a permeability barrier against hydrophobic substances and macromolecules (Nikaido, 1989). For this reason Gram-negative bacteria exhibit higher resistance to detergents and hydrophobic antibiotics than do Gram-positive bacteria (Nikaido, 1989). Due to its polycationic nature, PEI could be expected to act as an OM-permeabilizing agent. We have therefore

Abbreviations: NPN, 1-*N*-phenylnaphthylamine; OM, outer membrane; PEI, polyethyleneimine; PMBN, polymyxin B nonapeptide.

investigated the effect of PEI on different Gram-negative bacteria, focusing on phenomena associated with the permeability of the OM.

METHODS

Reagents. PEI (mean molecular mass 50 kDa), chicken egg white lysozyme (EC 3.2.1.17) and SDS were purchased from Sigma, and Triton X-100 from BDH.

Bacterial strains and growth conditions. The following strains were used: *Escherichia coli* ATCC 35150 (O157:H7), *Pseudomonas aeruginosa* ATCC 9027, *Salmonella typhimurium* ATCC 13311 and *Salmonella enteritidis* ATCC 13076. Unless otherwise stated, the growth medium in all liquid cultures was LB broth containing, per litre: 10 g Difco tryptone, 5 g Difco yeast extract, 5 g NaCl, pH 7.0. Cultivations were carried out at 37 °C with shaking (200 r.p.m.). Growth was monitored by measuring OD₆₃₀ with a Multiskan MCC/340 spectrophotometer (Labsystems).

Permeability assay based on the uptake of 1-N-phenyl-naphthylamine (NPN). The permeability of viable bacteria was measured by the uptake of the fluorescent hydrophobic probe NPN essentially as described by Loh *et al.* (1984). Briefly, the bacteria were cultivated to OD₆₃₀ 0.5, then the cells were deposited by centrifugation (1000 g, 10 min at room temperature) and suspended in a similar volume of 5 mM HEPES, pH 7.2. Samples (1.35 ml) of this suspension were pipetted directly into the fluorometer cuvette, and fluorescence was monitored with a Shimadzu RF-5000 spectrophotometer operated at wavelengths of 350 nm (excitation) and 420 nm (emission) and slit widths of 5 nm. After 30 s, NPN (0.5 mM in acetone) was added to a final concentration of 10 µM, and the resulting fluorescence was recorded. The test substance in aqueous solution was added, and the fluorescence again monitored. If desired, MgCl₂ was added to the cell suspension before addition of NPN.

Bacteriolysis assay. The bacteriolytic effect was assayed on Nunclon microtitre plates (Nunc) by measuring the OD₄₀₅ of bacterial cultures. First, the bacteria were grown to OD₆₃₀ 0.5, centrifuged as above, and suspended in a similar volume of 10 mM HEPES/50 mM NaCl. This suspension was divided into two portions, one of which was supplemented with PEI at 10 µg ml⁻¹. The suspensions were incubated at room temperature for 10 min, centrifuged and cells resuspended in the buffer without PEI. Aliquots (10⁸ cells in 100 µl) in quadruplicate were then pipetted into microtitre wells, which already contained appropriate amounts of lysozyme, Triton X-100, SDS or buffer only, and the OD₄₀₅ monitored. The degree of cell lysis was determined from the OD₄₀₅ values 4 min after the addition of cells. The value of the cell control without added lysozyme or detergent was taken as 100%; strong lysis is thus indicated by low percentage. Each assay was performed three times.

Antibiotic susceptibility and growth inhibition tests. The susceptibility of bacterial cultures to hydrophobic antibiotics was tested with the agar diffusion method on Iso-Sensitest agar (Oxoid) without or with PEI supplementation (50–500 µg ml⁻¹), using Neo-Sensitabs (erythromycin, novobiocin, clindamycin, fucidin and rifampicin; Rosco Diagnostica). The diameters of inhibition zones were measured after incubation of the plates at 37 °C overnight.

Further susceptibility tests with PEI and combinations of PEI and novobiocin were performed as described by Skyttä &

Mattila-Sandholm (1991) with an automated turbidimeter Bioscreen (Labsystems). A stock solution of novobiocin was prepared by dissolving 10 mg novobiocin (sodium salt, Sigma) in 70% (v/v) ethanol. The stock solution was further diluted (1:5) in distilled water, sterilized by filtration (0.22 µm) and used the same day.

The antimicrobial activity of PEI and novobiocin against *S. typhimurium* ATCC 13311 was studied by dispensing 30 µl of a 10⁻¹ dilution from a culture that had been grown in Iso-Sensitest broth (ISB, Oxoid) for 5 h (200 r.p.m., 37 °C) to microtitre plate wells with 270 µl test medium (ISB supplemented with PEI or PEI and novobiocin) and incubated at 37 °C for 24 h. The tested PEI concentrations were 2 and 10 µg ml⁻¹; novobiocin was tested at 10, 50 and 100 µg ml⁻¹. In the control sample wells, the antimicrobial agent was replaced by an equal volume of sterile water or ethanol. All determinations were replicated five times and results were expressed as mean values. The area under the growth curve was used as a measure of microbial growth, and the percentage reduction of area was used to express the growth inhibition in the presence of the antimicrobials.

Assay of liberated lipid by fatty acid analysis. *S. typhimurium* ATCC 13311 was grown in LB broth to OD₆₃₀ 0.6, and the culture was divided into three 10 ml portions, which were centrifuged to deposit the cells (3000 g, 30 min, 25 °C). The cells were washed with 10 mM Tris/HCl, pH 7.2, and again resuspended in 10 ml of the same buffer, which contained either EDTA (1 mM) or PEI (10 µg ml⁻¹). The control sample was suspended in buffer only. After 10 min at 37 °C the samples were centrifuged (11000 r.p.m. in an Eppendorf microfuge for 1 min at room temperature). Aliquots (10 µl) of the cell-free supernatants were taken for SDS-PAGE analysis, the rest were freeze-dried and 105 µg heptadecanoic acid methyl ester (Sigma) added to the dry samples, which were then subjected to fatty acid analysis. The samples were processed by saponification and methylation as described by Moore *et al.* (1994). The resulting fatty acid methyl esters were analysed with a HP5890 Series II (Hewlett-Packard) gas chromatograph equipped with a 7673A autosampler, a 25 m × 0.2 mm Ultra-2 column (Hewlett-Packard) and a flame ionization detector operated at 300 °C. The injector temperature was 250 °C. The temperature programme was from 170 °C to 260 °C at 5 °C min⁻¹ and then from 260 °C to 300 °C at 40 °C min⁻¹. Helium was used as carrier gas with a velocity of 27 cm s⁻¹. The split ratio was 1:50 and 1 µl was injected.

Miscellaneous methods. SDS-PAGE was carried out by the Laemmli (1970) system, using the Novex Xcell II apparatus and pre-cast 12% acrylamide gels. Lysates for SDS-PAGE were prepared and treated with proteinase K (Sigma) according to Hitchcock & Brown (1983).

RESULTS

Permeabilization assay by NPN fluorescence

NPN is a hydrophobic fluorescent probe whose quantum yield in hydrophobic environments is greatly increased as compared to hydrophilic environments, enabling its use in OM permeabilization studies (Träuble & Overath, 1973; Loh *et al.*, 1984; Hancock *et al.*, 1991; Tsuchido *et al.*, 1989; Freer *et al.*, 1996). We investigated the effect of PEI on NPN fluorescence in cell suspensions of *E. coli*, *P. aeruginosa* and *S. typhimurium*. Concentrations of PEI of up to 100 µg ml⁻¹ in

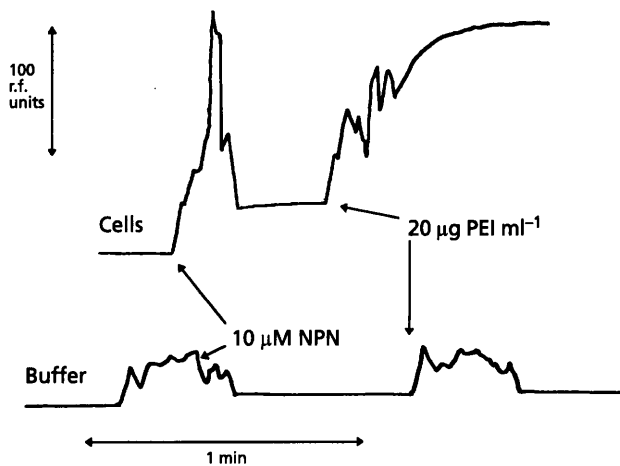


Fig. 1. Effect of addition of 10 μM NPN and 20 $\mu\text{g PEI ml}^{-1}$ into a suspension of *E. coli* (top) and into 5 mM HEPES buffer without bacteria (bottom). Fluorescence at 420 nm (excitation wavelength, 350 nm) was measured over time and expressed as relative fluorescence (r.f.) units. Sudden increases in fluorescence are due to opening of the cell chamber during addition of chemicals; the fluorescence values were obtained from stabilized curves.

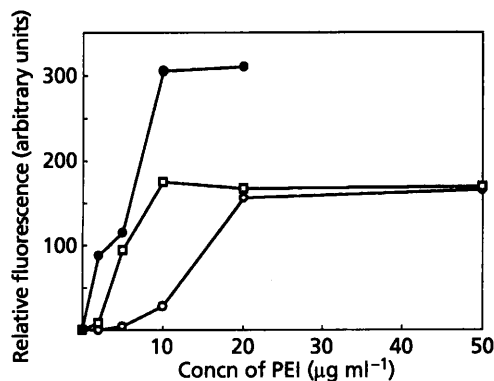


Fig. 2. Permeabilization of *E. coli* (○), *P. aeruginosa* (●) and *S. typhimurium* (□) by PEI, assayed by NPN uptake.

5 mM HEPES buffer containing NPN (10 μM) without bacteria caused no increase in fluorescence (Fig. 1). Fig. 1 also shows the result of a typical fluorescence assay in which NPN and PEI were sequentially added to a cell suspension of *E. coli*. Addition of NPN to the cell suspension always caused an increase in the fluorescence, and a prominent increase was observed after addition of PEI, exemplified in Fig. 1 by a concentration of 20 $\mu\text{g ml}^{-1}$. With *E. coli* the fluorescence increase was dose-dependent so that the relative fluorescence values increased proportionately up to 20 $\mu\text{g PEI ml}^{-1}$ (Fig. 2), whereas with *P. aeruginosa* and *S. typhimurium* a saturation in the uptake of NPN was observed already at 10 $\mu\text{g ml}^{-1}$ with cell suspensions adjusted to the same optical density as that of *E. coli* (Fig. 2). The highest levels of fluorescence were obtained with *P. aeruginosa*.

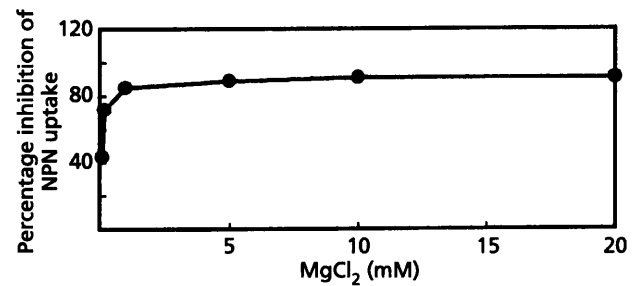


Fig. 3. Inhibition of PEI-induced permeabilization of *P. aeruginosa* by MgCl_2 assayed by NPN uptake. The PEI concentration was 5 $\mu\text{g ml}^{-1}$.

It was also noted that higher concentrations ($> 10 \mu\text{g ml}^{-1}$) of PEI resulted in the precipitation of bacterial cells, especially if the cultures had been centrifuged with refrigeration. Suspensions of *P. aeruginosa* were particularly prone to precipitation, and for this reason *P. aeruginosa* could not be tested with PEI concentrations higher than 20 $\mu\text{g ml}^{-1}$.

The well-known permeabilizer EDTA, which destabilizes the OM by chelating divalent cations (reviewed by Vaara, 1992), was tested in parallel with PEI. With each of the three bacterial species mentioned above, 2 mM EDTA caused fluorescence increases that did not differ from the maximal levels induced by PEI (data not shown), indicating that these two substances possess similar permeabilization potencies in the conditions employed.

Since divalent cations such as Mg^{2+} are known to inhibit the action of many OM permeabilizers that act by either chelating or replacing cations in the OM (Vaara, 1992), we tested whether Mg^{2+} affects the activity of PEI. Fig. 3 shows the inhibitory effect of added MgCl_2 on the NPN uptake in the presence of 5 $\mu\text{g PEI ml}^{-1}$ by *P. aeruginosa*. Even at submillimolar concentrations Mg^{2+} had an inhibitory effect on NPN uptake, the maximum inhibition remaining at 90% within the concentration range tested. NPN uptake in the presence of Mg^{2+} reached a constant value after a period of typically less than 30 s. For PEI-induced NPN uptake by *S. typhimurium* and *E. coli*, the inhibition by Mg^{2+} was less prominent and exhibited different kinetics from that observed with *P. aeruginosa*, in that Mg^{2+} only slowed the uptake of NPN. Thus in the presence of Mg^{2+} a retarded but constantly increasing fluorescence was observed, as shown in Fig. 4 for *E. coli* with 20 $\mu\text{g PEI ml}^{-1}$ and 1 mM MgCl_2 . For *S. typhimurium*, tested with 5 $\mu\text{g PEI ml}^{-1}$, Mg^{2+} exhibited a clear-cut inhibitory effect in concentrations higher than 1 mM.

Effect of PEI on antibiotic susceptibility

A sensitizing effect to hydrophobic antibiotics is one of the indications of OM-permeabilizing action (Vaara, 1992). We tested the susceptibility of *E. coli*, *P. aeru-*

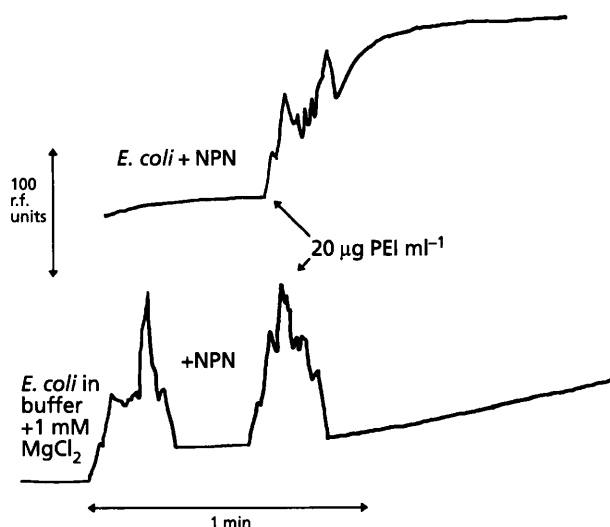


Fig. 4. Uptake of NPN by suspensions of *E. coli* induced by $20 \mu\text{g ml}^{-1}$ PEI in the absence (top) and presence (bottom) of 1 mM MgCl_2 .

Table 1. Effect of PEI on the susceptibility of *E. coli*, *P. aeruginosa* and *S. typhimurium* to selected antibiotics determined by the agar diffusion method

Antibiotic	Diameter of inhibition zone (mm) on plated cultures			
	PEI concn ($\mu\text{g ml}^{-1}$):			
	0	50	200	500
<i>E. coli</i>				
Erythromycin	17	18	19	18
Novobiocin	12	15	25	27
Clindamycin	0	17	17	20
Fucidin	0	0	20	25
Rifampicin	23	25	25	30
<i>P. aeruginosa</i>				
Erythromycin	19	20	17	23
Novobiocin	0	0	20	45
Clindamycin	0	0	0	15
Fucidin	0	0	0	22
Rifampicin	17	19	19	39
<i>S. typhimurium</i>				
Erythromycin	16	16	21	24
Novobiocin	0	15	22	30
Clindamycin	0	0	16	16
Fucidin	0	0	13	21
Rifampicin	22	22	24	30

ginosa and *S. typhimurium* to a set of hydrophobic antibiotics (erythromycin, novobiocin, clindamycin, fucidin and rifampicin) by the agar diffusion method on plates containing different concentrations of PEI. The results are summarized in Table 1, which shows that PEI

Table 2. Synergistic inhibition of growth of *S. typhimurium* by PEI and novobiocin

The results are means of five determinations \pm SD.

Novobiocin concn ($\mu\text{g ml}^{-1}$)	Growth inhibition (%) in 24 h		
	PEI concn ($\mu\text{g ml}^{-1}$):		
	0	2	10
0	0	4.8 ± 4.5	8.8 ± 4.3
10	0	0	40.6 ± 3.0
30	0	0	61.2 ± 0.9
100	1.7 ± 1.5	22.6 ± 9.9	63.4 ± 6.8

induced clear-cut susceptibility to novobiocin, clindamycin and fucidin, these antibiotics being ineffective towards the test bacteria in the absence of PEI. However, on agar plates much larger concentrations ($> 50 \mu\text{g ml}^{-1}$) were required for sensitization to antibiotics than for NPN uptake in suspension ($10 \mu\text{g ml}^{-1}$). There was only weak, if any, sensitizing action for erythromycin, and for rifampicin the sensitizing effect was observed at $500 \mu\text{g PEI ml}^{-1}$, most clearly for *P. aeruginosa*.

The effect of PEI on growth of *S. typhimurium* was also tested using the automated turbidimeter Bioscreen, where a definite sensitizing effect to novobiocin was observed (Table 2). In this experiment, PEI alone at concentrations of 2 and $10 \mu\text{g ml}^{-1}$ brought about only negligible growth inhibition of *S. typhimurium*. Essentially similar results were obtained for *E. coli* and *P. aeruginosa* with combinations of PEI and novobiocin. Furthermore, in Bioscreen assays concentrations of up to $500 \mu\text{g PEI ml}^{-1}$ alone caused no significant growth inhibition of *E. coli*, *P. aeruginosa* or *S. typhimurium*.

Sensitization to lytic action of lysozyme and detergents

Permeabilization of the OM is also manifested as an increased susceptibility to the bacteriolytic action of detergents and, in some cases, lysozyme (Vaara, 1992). We therefore tested the effect of PEI on the action of lysozyme and the detergents SDS and Triton X-100 on *E. coli*, *P. aeruginosa* and *S. typhimurium*. In these assays, cells were pretreated with PEI and subsequently washed so that PEI was not present in the assay medium. The results are shown in Table 3. Lysozyme ($10 \mu\text{g ml}^{-1}$) exhibited no lytic activity towards the untreated cells of any of the three species. With *P. aeruginosa* a weak lysis was observed in the PEI-treated culture, whereas cultures of *E. coli* and *S. typhimurium* remained unaffected. Only *P. aeruginosa* was sensitized by PEI to Triton X-100. All of the test species were, however, strongly sensitized to SDS. Experiments with *S. typhimurium* were also carried out in which PEI was present together with lysozyme, SDS or Triton X-100 in the microtitre plate wells. In these tests no sensitization to SDS was

Table 3. Effect of PEI on bacteriolysis

The degree of cell lysis was determined from the OD₄₀₅ values 4 min after the addition of cells. The value of the cell control without added lysozyme or detergent was taken as 100%; strong lysis is thus indicated by a low percentage. Each assay was performed three times with (+PEI) or without (-PEI) pretreatment with 10 µg PEI ml⁻¹. Figures in parentheses are SD.

Lytic agent	Relative turbidity (%)					
	<i>E. coli</i>		<i>P. aeruginosa</i>		<i>S. typhimurium</i>	
	-PEI	+PEI	-PEI	+PEI	-PEI	+PEI
Lysozyme (10 µg ml ⁻¹)	100 (1)	101 (1)	100 (2)	92 (7)	100 (2)	101 (3)
SDS (0.1%, w/v)	100 (2)	38 (8)	102 (3)	31 (19)	64 (2)	23 (2)
SDS (1%, w/v)	92 (1)	35 (10)	93 (4)	24 (18)	21 (4)	22 (2)
Triton X-100 (0.1%, w/v)	102 (1)	102 (1)	100 (3)	78 (6)	101 (2)	101 (1)
Triton X-100 (1%, w/v)	92 (1)	92 (1)	95 (5)	62 (12)	88 (1)	89 (3)

Table 4. Assay of fatty acids derived from cell-free supernatants of *S. typhimurium* cultures treated with EDTA or PEI

Fatty acid	Concn [µg (ml cell-free supernatant) ⁻¹]		
	Untreated	+EDTA (1 mM)	+PEI (10 µg ml ⁻¹)
12:0	0.09	0.37	0.05
14:0	0.11	0.60	<0.02
16:0	0.36	1.53	0.10
18:0	<0.02	<0.02	<0.02
17:0 cyc*	<0.02	0.20	<0.02
16:1	0.18	0.83	<0.02
18:1	0.19	1.01	<0.02
14:0 (3-OH)	0.22	1.28	<0.02
Sum of quantified fatty acids	1.15	5.82	0.15

* cyc, cyclopropane.

observed. Instead, a weak synergistic effect was noted for combinations of PEI with either lysozyme or Triton X-100 (data not shown).

Does PEI liberate LPS?

Permeabilizing agents such as chelators and certain polycations release LPS from the OM, whereas others such as polymyxin B nonapeptide (PMBN) cause OM permeabilization without significant liberation of LPS (Vaara, 1992). To investigate the mechanism of action of PEI upon *S. typhimurium*, we treated bacterial suspensions with PEI and in parallel with EDTA in Tris buffer, and analysed the material remaining in the cell-free supernatants by fatty acid analysis to reveal LPS-specific fatty acids indicating liberation of LPS from the OM. Table 4 shows the amounts of fatty acids found in the supernatants after saponification and methylation. Fatty acids typical of *S. typhimurium* cells were present

in the control sample, indicating some release of membrane components to the medium. In the EDTA-treated sample the total amount of fatty acids was several times higher, and the proportion of the LPS-specific 3-hydroxytetradecanoic acid was approximately six times larger than that found in the control sample. This indicates that EDTA had liberated LPS from the cells. In the PEI-treated sample, the amount of all fatty acids was very low, showing that the supernatants contained no detectable LPS. This was confirmed by SDS-PAGE, as no LPS could be visualized in gels by silver staining in lanes loaded with proteinase-K-treated supernatants of PEI supernatants. Instead, samples of EDTA supernatants revealed ladder-like patterns typical of smooth-type LPS (not shown). It is apparent from this experiment that upon treatment with PEI no LPS was liberated from the cells in a soluble form. The possibility still exists, however, that PEI released LPS from the OM, but due to the water-insoluble nature of the PEI-LPS

complex it could not be detected in the cell-free supernatant.

DISCUSSION

On the basis of our results it can be concluded that even at sub-inhibitory concentrations PEI makes Gram-negative bacteria permeable to hydrophobic probes such as antibiotics and NPN. Sensitization of Gram-negative bacteria to hydrophobic antibiotics is a property shared by all polycationic permeabilizers such as deacylated polymyxin derivatives containing a cyclic heptapeptide, e.g. PMBN (reviewed by Vaara, 1992), polylysine and protamine (Vaara & Vaara, 1983a). Of these agents, the latter two polycations release considerable proportions of LPS from the OM, whereas PMBN acts in a different manner, binding to LPS but not releasing it to the medium (Vaara & Vaara, 1983b). Bacteria are known not to be sensitized to the anionic detergent SDS when tested together with the above permeabilizers (Vaara & Vaara, 1983a), as was also shown for PEI. It can be assumed that the large polycation PEI interacts electrostatically with SDS and probably sequesters it to an ineffective form. However, PEI did strongly sensitize all test bacteria to SDS in our assay in which cells were pretreated with PEI. This shows that PEI was able to disorganize the OM not only transiently but in an irreversible manner in these conditions.

No sensitization to lysozyme was observed with PEI, in accordance with the similar inability of other polycationic permeabilizers. The permeation of lysozyme apparently requires extensive disorganization in the OM, such as that resulting from the massive loss of LPS from the OM by EDTA, as first shown by Leive (1965). We could not demonstrate liberation of LPS from *S. typhimurium* by PEI. PEI is known to bind to and precipitate various cellular materials such as lipids and nucleic acids (Salt *et al.*, 1995). Since LPS molecules are normally anionic due to the presence of phosphate and carboxyl groups (Helander *et al.*, 1996), PEI can be expected to bind strongly to LPS, forming insoluble complexes that make it impossible to measure LPS liberation by investigating cell-free supernatants after centrifugation of PEI-treated cultures. At present it seems most probable that PEI disorganizes the OM by intercalating in the LPS layer similarly to PMBN. The interactions of PEI and LPS certainly warrant further study, and since PEIs are available in different molecular sizes and forms, it would be of interest to determine their properties with respect to interactions with bacterial surface components. It would also be interesting to test the ability of PEI to permeabilize Gram-positive bacteria and eukaryotic cells; in fact PEI has been shown to affect neutrophil plasma membranes, resulting in the leakage of enzymes from the cells (Elferink, 1991).

Although a potent permeabilizer, PEI did not inhibit the growth of *E. coli*, *P. aeruginosa* or *S. typhimurium* to any significant extent. This is in agreement with Tashiro (1992), who showed that PEI600 had a small bacteriostatic effect on *E. coli* and *P. aeruginosa*.

By virtue of its permeability-increasing properties, PEI can be expected to act as a vehicle for experimental drug delivery into cells, and as a potentiating agent for antibacterial agents that are normally excluded by Gram-negative bacteria. Although PEI has a good safety record, further studies will be required to evaluate its applicability in areas such as disinfection and food protection.

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REFERENCES

- Bahulekar, R., Ayyangar, N. R. & Ponrathnam, S. (1991). Polyethyleneimine in immobilization of biocatalysts. *Enzyme Microb Technol* 13, 858-868.
- Cong, L., Kaul, R., Dissing, U. & Mattiasson, B. (1995). A model study on Eudragit and polyethyleneimine as soluble carriers of α -amylase for repeated hydrolysis of starch. *J Biotechnol* 42, 75-84.
- Cordes, R. M., Sims, W. B. & Glatz, C. E. (1990). Precipitation of nucleic acids with poly(ethyleneimine). *Biotechnol Prog* 6, 283-285.
- Elferink, J. G. R. (1991). Changes of plasma membrane permeability in neutrophils treated with polycations. *Inflammation* 15, 103-115.
- Freer, E., Moreno, E., Moriyón, I., Pizarro-Cerdá, J., Weintraub, A. & Gorvel, J.-P. (1996). *Brucella-Salmonella* lipopolysaccharide chimeras are less permeable to hydrophobic probes and more sensitive to cationic peptides and EDTA than are their native *Brucella* sp. counterparts. *J Bacteriol* 178, 5867-5876.
- Hancock, R. E. W., Farmer, S. W., Li, Z. & Poole, K. (1991). Interaction of aminoglycosides with the outer membranes and purified lipopolysaccharide and OmpF porin of *Escherichia coli*. *Antimicrob Agents Chemother* 35, 1309-1314.
- Helander, I. M., Mäkelä, P. H., Westphal, O. & Rietschel, E. T. (1996). Lipopolysaccharides. In *Encyclopedia of Molecular Biology and Molecular Medicine*, vol. 3, pp. 462-471. Edited by R. A. Meyers. Weinheim: VCH.
- Hitchcock, P. J. & Brown, T. M. (1983). Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. *J Bacteriol* 154, 269-277.
- Kirk, N. & Cowan, D. (1995). Optimising the recovery of recombinant thermostable proteins expressed in mesophilic hosts. *J Biotechnol* 42, 177-184.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680-685.
- Leive, L. (1965). Release of lipopolysaccharide by EDTA treatment of *E. coli*. *Biochem Biophys Res Commun* 21, 290-296.
- Loh, B., Grant, C. & Hancock, R. E. W. (1984). Use of the fluorescent probe 1-N-phenyl-naphthylamine to study the interactions of aminoglycoside antibiotics with the outer membrane of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 26, 546-551.
- Milburn, P., Bonnerjea, J., Hoare, M. & Dunnill, P. (1990). Selective flocculation of nucleic acids, lipids, and colloidal particles from a yeast cell homogenate by polyethyleneimine, and its scale-up. *Enzyme Microb Technol* 12, 527-532.
- Moore, L. V. H., Bourne, D. M. & Moore, W. E. C. (1994). Comparative distribution and taxonomic value of cellular fatty acids

in thirty-three genera of anaerobic Gram-negative bacilli. *Int J Syst Bacteriol* **44**, 338–347.

Nikaido, H. (1989). Outer membrane barrier as a mechanism of antimicrobial resistance. *Antimicrob Agents Chemother* **33**, 1831–1836.

Salt, D. E., Hay, S., Thomas, O. R. T., Hoare, M. & Dunnill, P. (1995). Selective flocculation of cellular contaminants from soluble proteins using polyethyleneimine: a study of several organisms and polymer molecular weights. *Enzyme Microb Technol* **17**, 107–113.

Skyttä, E. & Mattila-Sandholm, T. (1991). A quantitative method for assessing bacteriocins and other food antimicrobials by automated turbidometry. *J Microbiol Methods* **14**, 77–88.

Suh, J., Cho, Y. & Lee, K. J. (1991). Macrocyclic metal complexes built on polyethyleneimine. *J Am Chem Soc* **113**, 4198–4202.

Tashiro, T. (1992). Removal of bacteria from water by systems based on insoluble polystyrene–polyethylenimine. *J Appl Polym Sci* **46**, 899–907.

Träuble, H. & Overath, P. (1973). The structure of *Escherichia coli* membranes studied by fluorescence measurements of lipid phase transitions. *Biochim Biophys Acta* **307**, 491–512.

Tsuchido, T., Aoki, I. & Takano, M. (1989). Interaction of the fluorescent dye 1-N-phenyl-naphthylamine with *Escherichia coli* cells during heat stress and recovery from heat stress. *J Gen Microbiol* **135**, 1941–1947.

Vaara, M. (1992). Agents that increase the permeability of the outer membrane. *Microbiol Rev* **56**, 395–411.

Vaara, M. & Vaara, T. (1983a). Polycations sensitize enteric bacteria to antibiotics. *Antimicrob Agents Chemother* **24**, 107–113.

Vaara, M. & Vaara, T. (1983b). Polycations as outer membrane-disorganizing agents. *Antimicrob Agents Chemother* **24**, 114–122.

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