Increases in permeability of *Escherichia coli* outer membrane induced by polycations

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The action of polycations (such as polylysine and compound 48/80) on *Escherichia coli* was studied with use of Ca^{2+}, K^+ and TPP^+ ion-selective electrodes. Rapid efflux of Ca^{2+} was observed when a polycation was added in cell suspension. The polycation treatment promoted a drug-inducing K^+ release from the cytoplasmic membrane. TPP^+ uptake was also increased by addition of a polycation. Without the polycation treatment, the uptake of TPP^+ was largely suppressed due to a permeability barrier of the outer membrane. The results show that a polycation disrupted the permeability barrier of the outer membrane.

Polycation *Polylysine* *Compound 48/80* *Ion-selective electrode* *Outer membrane* *Membrane permeability*

1. INTRODUCTION

Gram-negative bacteria have an outer membrane in their cell structure. Many hydrophobic antibiotics or macromolecular proteins (such as lysozyme) cannot permeate through the outer membrane [1]. This permeability barrier of the outer membrane is disrupted when cells are treated with EDTA [1]. It is now well established that LPS is located at the outside of the outer membrane, and divalent cations (e.g. Ca^{2+} and Mg^{2+}) maintain the outer membrane structure [2]. About half as much as LPS was released by the EDTA treatment from the outer membrane [1,3]. EDTA removes Ca^{2+} and other divalent cations from the membrane, and this removal presumably triggers the release of LPS and the disruption of the permeability barrier of the outer membrane.

Polymyxin, known as a polycationic amphipathic antibiotics, binds strongly to LPS, and also disrupts the permeability barrier of the outer membrane [2,4]. It seems likely that divalent cations at the binding sites are replaced by polymyxin molecules. We have found that polymyxin treatment induced Ca^{2+} release from *E. coli* cells leading to disruption of the outer membrane structure [5]. This result prompted us to investigate the action of other polycations on the outer membrane. It was found that various polycations (such as polylysine and compound 48/80) released Ca^{2+} from *E. coli* cells and promoted gramicidin-induced K^+ permeability of the cytoplasmic membrane. An increase in TPP^+ uptake was also observed after polycation treatment. These results show that polycations disrupted the permeability barrier of the outer membrane.

Abbreviations: TPP^+, tetraphenylphosphonium ion; LPS, lipopolysaccharide; PVC, polyvinyl chloride; Mops, 4-morpholinepropanesulphonic acid; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone

2. MATERIALS AND METHODS

The changes in ion fluxe of K^+, Ca^{2+} and TPP^+ were measured with ion-selective electrodes (ISEs). These ISEs were constructed by the use of PVC-based membranes as in [6–10]. PVC membranes had the following composition: (a) K^+ ISE [6–8], 1 mg valinomycin, 50 μl di(*n*-octyl)sebacate and 25 mg PVC; (b) Ca^{2+} ISE [8], 0.9 mg *N,N'*-di(11-ethoxycarbonylundecyl)-*N,N'*-4,5-tetramethyl-3,6-dioxaoctane diamide (Ca^{2+} neutral carrier, ETH 1001), 65 μl *o*-nitrophenyl *n*-octyl ether and 35 mg

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PVC; (c) TPP^+ ISE [9,10], 0.1 mg sodium tetraphenylborate, 60 μl di(*n*-octyl)phthalate and 25 mg PVC. The materials dissolved in tetrahydrofuran were poured into a flat Petri dish of 30 mm diameter, and then the solvent was evaporated off at room temperature. The resulting PVC membrane was used as a sensor membrane. The large interference of compound 48/80 with the Ca^{2+} ISE did not permit precise determination of Ca^{2+} release by this compound.

The bacterial strain used in this study was *E. coli* W3133, a derivative of K12. The bacteria were grown at 37°C in minimal salt medium [11] supplemented with 1% polypeptone. Cells were harvested in the late exponential phase of growth, washed twice with buffer (50 mM Mops-Tris and 100 mM choline chloride, pH 7.2), and suspended in this buffer at 1 mg cell protein/ml. Protein content was determined as in [12].

3. RESULTS AND DISCUSSION

Fig. 1 shows Ca^{2+} release upon addition of poly(L-lysine) (degree of polymerization = 20). The Ca^{2+} release was observed immediately after addition of polylysine. The addition of L-lysine (monomer) did not induced any Ca^{2+} release. The Ca^{2+} release implies disruption of the outer membrane structure, since divalent metal ions (such as Ca^{2+} and Mg^{2+}) are essential to maintain the membrane structure [2]. Fig. 2 shows the temperature dependence of Ca^{2+} release. A rapid efflux of Ca^{2+} was observed irrespective of the temperature. Authors in [3] reported that LPS, existing in the outer membrane, was released when *E. coli* cells were treated with EDTA, and the rates of the release did not vary at 37 and 0°C. The EDTA treatment causes elimination of Ca^{2+} and other divalent cations from the outer membrane, triggering the release of LPS and disruption of the permeability barrier of the outer membrane. The Ca^{2+} release by polylysine and its temperature-insensitive nature suggest that polylysine treatment also disrupted the barrier function of the outer membrane.

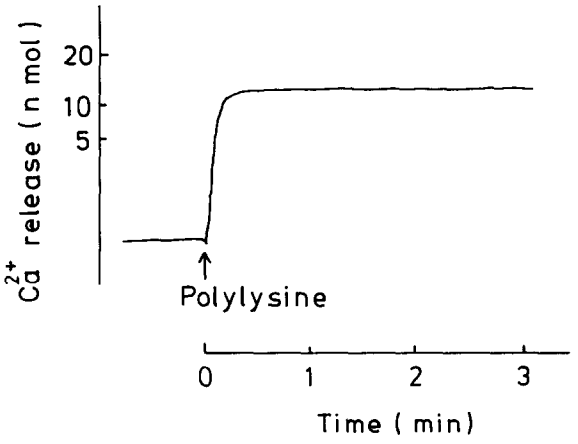

Fig. 1. Ca^{2+} release from *E. coli* cells upon addition of polylysine. Cell suspension (0.1 ml, 0.1 mg cell protein) was added to buffer solution (2.0 ml) containing 50 mM Mops-Tris (pH 7.2) and 100 mM choline chloride. At arrow, 50 μl polylysine (final concentration: 25 μg/ml) was added.

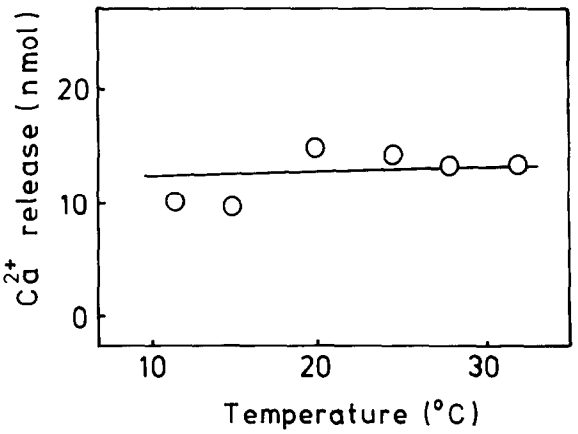

Fig. 2. Temperature dependence of Ca^{2+} release from *E. coli* cells. The amount of release was determined from the Ca^{2+} efflux within 2 min after polylysine addition.

To determine more directly the effect of polylysine on the outer membrane, we investigated the synergism of polylysine on the action of gramicidin, a channel-forming ionophore [13]. The addition of gramicidin alone to cells was less effective due to the barrier function of the outer membrane. If gramicidin molecules reach the cytoplasmic membrane, pores of diameter 4 Å are formed and thus K^+ is released from the cytoplasmic membrane. Fig. 3 shows an increase in gramicidin-induced K^+ permeability upon addition of polylysine at 37°C. Without polylysine, K^+ release did not take place at this concentration of gramicidin.

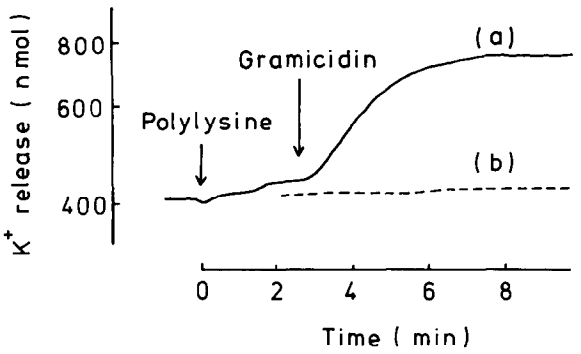

Fig. 3. Gramicidin-induced K^+ permeability change (37°C); (a) and (b) results with and without addition of polylysine, respectively. Cells (0.1 ml, 0.1 mg cell protein) were added to 2.0 ml of 50 mM Mops-Tris (pH 7.2) and 100 mM choline chloride. At the first arrow 50 μl polylysine (final concentration: 25 μg/ml) was added [(a) only]. Gramicidin (final concentration: 1 μM) was added at the second arrow [both (a) and (b)].

The following experiment further demonstrated the disruption of the permeability barrier by polylysine treatment. It is known that the lipid-soluble cation, TPP^+, diffuses passively across the membrane depending on the membrane potential [9,10]. Such diffusion of TPP^+ does not to a great extent occur without EDTA treatment of cells [14]. The barrier function of the outer membrane hinders penetration of TPP^+ into the cells. Fig. 4 shows an increase in TPP^+ uptake upon addition of polylysine. At zero time, an *E. coli* suspension was added to buffer solution containing 10^{-5} M TPP^+ and 10 mM sodium lactate. A small TPP^+ uptake was observed. When polylysine was added in this suspension, the TPP^+ uptake was largely promoted, indicating that polylysine disrupted the barrier function of the outer membrane. Addition of an uncoupler, FCCP, brought about the efflux of the accumulated TPP^+. FCCP abolished completely the membrane potential.

Although our experiments demonstrate that polylysine disrupted the permeability barrier of the outer membrane, we found that another polycation, compound 48/80 [7], also enhanced gramicidin-induced K^+ permeability and TPP^+ uptake, similar to the action of polylysine. Thus, we conclude that the disruptive action of a polycation on barrier function took place irrespective of the kind of polycation.

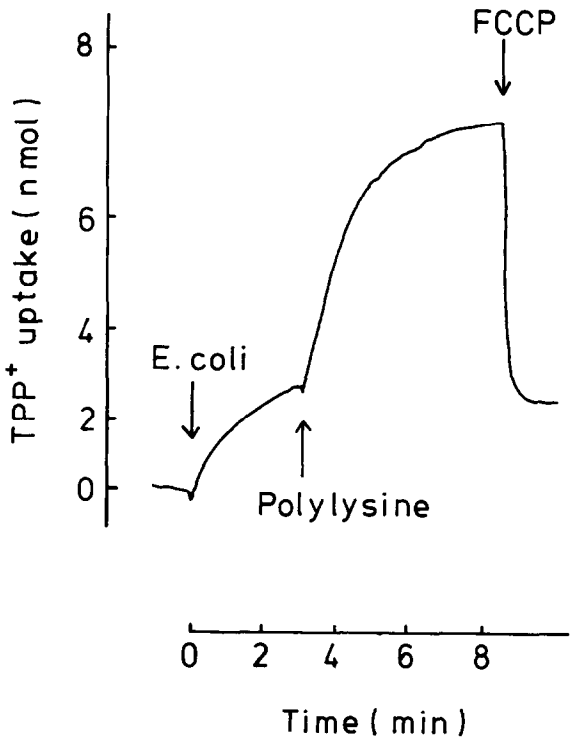

Fig. 4. Uptake of TPP^+ after addition of polylysine. Assay mixtures consisted of 1.0 ml of 50 mM Mops-Tris (pH 7.2), 100 mM choline chloride, 10 mM sodium lactate, and 10^{-5} M TPPCl. At the first arrow, 50 μl cell suspension (0.05 mg cell protein) was added. The second arrow indicates the time when 25 μl polylysine (final concentration: 25 μg/ml) was added, and at the third arrow FCCP (final concentration: 1 μM) was added.

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