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DISSIPATION OF MEMBRANE POTENTIAL OF *Escherichia coli* CELLS INDUCED BY MACROMOLECULAR POLYLYSINE

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SUMMARY: Macromolecular polylysine caused a rapid and drastic decrease in the membrane potential of *Escherichia coli* cells, monitored with the uptake of the lipophilic cation, tetraphenylphosphonium cation (TPP^+). Respiration of cells was not affected to a significant extent by polylysine. Polylysine treatment induced the efflux of K^+ from cells, suggesting that an increase in permeability of cytoplasmic membrane may be responsible for the decrease in the membrane potential. The action of polylysine is discussed in comparison with that of colicin E1 which is known to dissipate the membrane potential of *E. coli* cells.

Polylysine inhibits growth of Gram-negative bacteria [1]. This inhibitory effect is dependent on the degree of polymerization (n) of polylysine [2]. Highly polymerized polylysines inhibit cell growth more strongly. We were interested in the recent observations that various polycations including polylysine rendered a bacterial outer membrane permeable to several drugs [2-5]. It is probable that polylysine attacks an inner cytoplasmic membrane of bacteria after disruption of the outer membrane structure.

The present study examined the effect of polylysine on the membrane of *Escherichia coli* by using various degrees of polymerization ($n = 20 \sim 700$) of polylysine. It was found that polylysines with $n > 100$ dissipated the membrane potential. The efflux of K^+ could also be observed after polylysine addition. The results suggest that polylysine affects the permeability of cytoplasmic membrane leading to dissipation of the membrane potential.

Abbreviations: TPP^+, tetraphenylphosphonium ion; Mops, 4-morpholine-propanesulphonic acid; Tris, tris(hydroxymethyl)aminomethane; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone

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MATERIALS AND METHODS

Poly(L-lysine) hydrobromides were purchased from Sigma Chemical Co. The amounts of TPP^+ uptake and K^+ efflux were measured with ion-selective electrodes (ISEs). These ISEs were constructed by the use of poly(vinyl chloride)-based membranes as reported [4-6].

The bacterial strains used in this study were *E. coli* W3110, a derivative of K 12, and AS-1, an *acr*A mutant derived from W3110 [7]. Cells were grown at 37°C in a minimal salt medium [8] supplemented with 1% polypeptone. Preparative conditions of cells were the same as those reported previously [4,5]. Protein content was determined by the Lowry's method [9].

RESULTS AND DISCUSSION

We investigated the effect of polylysine on the membrane potential by monitoring the uptake of TPP^+. It is known that TPP^+ diffuses passively across membrane depending on the membrane potential [10,11]. Such diffusion does not efficiently occur in the case of Gram-negative bacteria, due to the presence of an outer membrane [12]. Polylysine ($n = 20$) treatment enabled TPP^+ to pass through cells [5], suggesting that polylysine disrupted the barrier function of the outer membrane. Here we investigated the effect of the degree of polymerization of polylysine on the amount of the uptake of TPP^+ (Fig. 1). The uptake of TPP^+ was remarkably decreased by addition of the higher degrees of polymerization of polylysines ($n > 100$). It was conceivable that these polylysines ($n > 100$) attacked the cytoplasmic membrane to dissipate the membrane

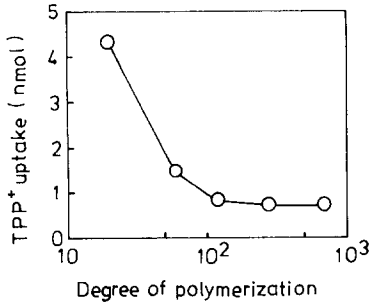

Fig. 1. Effect of the degree of polymerizatin of polylysine on the uptake of TPP^+. *E. coli* strain used was W3110. Cell suspension (0.05 ml, 0.5 mg cell protein) was diluted in 1 ml of assay mixture containing 50 mM Mops-Tris (pH 7.2), 100 mM choline chloride, 10 mM sodium lactate, and 10 μM TPPCl at 26°C. Then, 25 μl polylysine (final concentration: 25 μg/ml) was added, and 2 min later FCCP (final concentration: 1 μM) was added. The uptake of TPP^+ was estimated from the amount effluxed by FCCP addition.

potential. In accordance with the decrease in TPP^+ uptake, polylysines with n > 100 inhibited cell growth (data not shown) as reported by Vaara and Vaara [2].

It was also thought, however, that highly polymerized polylysines adsorbed on cell surface might hinder simply the penetration of TPP^+ into cells due to electrostatic repulsion. To test whether or not polylysine induces the dissipation of the membrane potential, we utilized a lipopolysaccharide-defective mutant (AS-1) of *E. coli* [7]. This mutant incorporates spontaneously TPP^+ into cells [11], and thus the effect of polylysine on the membrane potential can be evaluated by measuring the efflux of the accumulated TPP^+. It was observed that highly polymerized polylysine with n = 280 (at 25 μg/ml) caused efflux of the accumulated TPP^+ (Fig. 2). Addition of polylysine with n = 120 also caused efflux of TPP^+, while addition of polylysine with n = 20 had no effect (data not shown). Respiration of cells was not affected to a significant extent at this concentration of polylysine, indicating that dissipation of the membrane potential was not induced by inhibition of the respiratory chain. A possible cause for the decrease in the membrane potential is a change in permeability of the cytoplasmic membrane. To evaluate this possibility,

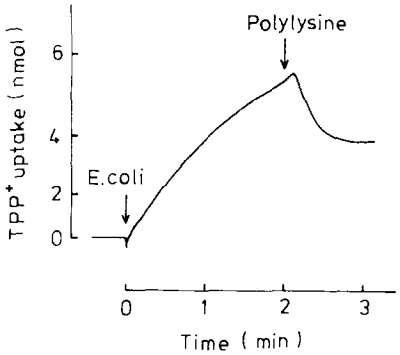

Fig. 2. Efflux of accumulated TPP^+ caused by addition of highly polymerized polylysine. Assay mixture was the same as in Fig. 1, except that AS-1 strain was used. At the time indicated by the first arrow, 50 μl of cell suspension (0.5 mg cell protein) was added. The second arrow indicates the time when 25 μl of polylysine with n = 280 (final concentration: 25 μg/ml) was added.

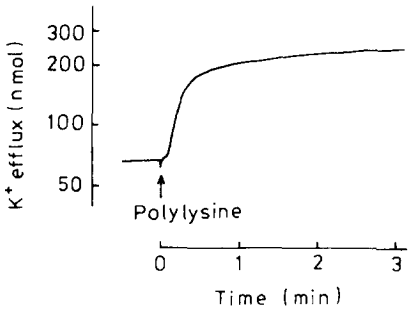

Fig. 3. Efflux of K^+ caused by addition of polylysine. AS-1 strain was used. Cell suspension (0.05 ml, 0.5 mg cell protein) was diluted in 1 ml of assay mixture containing 50 mM Mops-Tris (pH 7.2), 100 mM choline chloride, and 10 mM sodium lactate at 26°C. At the time indicated by the arrow, 25 µl of polylysine with n = 280 (final concentration: 25 µg/ml) was added.

we measured a change in the K^+ permeability after polylysine treatment. The efflux of K^+ was greatly stimulated by addition of polylysine with n = 280 (Fig. 3). Similar results were obtained with both W3110 and AS-1. This efflux was not energy-dependent, since addition of FCCP, a potent H^+ conductor, did not affect the efflux of K^+. Addition of FCCP alone did not cause efflux of K^+, indicating that dissipation of the membrane potential did not trigger the permeability change. These results suggest that an increase in permeability of cytoplasmic membrane induced by polylysine elicited the decrease in the membrane potential.

Several reasons were considered for the permeability change. After polylysine molecules disrupted the permeability barrier of the outer membrane [2,3,5], they may diffuse to the cytoplasmic membrane to induce the permeability change. If this is the case, it seems likely that polylysine treatment increases the permeability of isolated cytoplasmic membrane vesicles [13] to abolish its membrane potential. However, polylysine did not dissipate the membrane potential of isolated membrane vesicles. We compared the action of polylysine with that of colicin E1 which is known to inhibit energy metabolism in a sensitive *E. coli* cells [14]. It should be pointed out that colicin E1 is rich in lysine residue (60 out of 522 residues) [15]. Many data have shown that macromolecular colicin E1 adsorbed on the specific receptor of the outer membrane and

caused the membrane depolarization [14]. The action of colicin E1 was energy-dependent. Colicin E1 also acted on liposomes, suggesting that it penetrated the cytoplasmic membrane to dissipate the membrane potential [16]. In contrast, polylysine did not require cellular energy to increase the permeability of the cytoplasmic membrane. It might be possible that polylysine reaches inner membrane by energy-independent process. However, because polylysine is a highly polar protein, its penetration through the cytoplasmic membrane seems unlikely [4]. The mechanism by which polylysine changed the permeability is not clear at present. It may also be possible that polylysine interacts with the outer membrane of cells, leading to a change in the K^+ permeability and dissipation of the membrane potential by unknown mechanism.

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