INTERACTION BETWEEN THE ESCHERICHIA COLI OUTER MEMBRANE PROTEIN F AND THE ANTIBIOTIC CEFTAZIDIME

Adela Banciu, *CONSTANTIN BRÂNCUȘI UNIVERSITY OF T*ÂRGU JIU, TÂRGU JIU, ROMANIA
George Necula, INCDMI CANTACUZINO, BUCHAREST, ROMANIA
Beatrice Mihaela Radu, UNIVERSITY OF BUCHAREST, BUCHAREST, ROMANIA
Daniel Dumitru Banciu, UNIVERSITY OF BUCHAREST, BUCHAREST, ROMANIA

ABSTRACT: The electrophysiological tip-dip technique allows the identification of cell behaviors that depend on many factors, such as antibiotic resistance. These properties of membrane allow the development of devices capable of identifying antibiotic resistance in a manner faster than traditional screening tests for antibiotic resistance. In this way, the doctors can use the most appropriate antibiotic, with decreased occurrence of antibiotic resistance.

KEY WORDS: tip-dip technique, outer membrane protein F, ceftazidime, antibiotic resistance

1. INTRODUCTION

OmpF (outer membrane protein F) belongs to a class of membrane proteins, porins, forming membrane channels in Gram-negative bacteria. Gram-negative bacteria such as Escherichia coli have an outer membrane, composed of phospholipids, proteins and lipopolysaccharides [1], which protects the bacteria against different environmental conditions and acts as a passive filter for maintaining the influx and efflux of substances [2]. This function is performed by water channel-forming transmembrane proteins (porins) that facilitate passive transport of ions and nutrients into the bacterial periplasmic space. The most studied porins of E. coli are outer membrane protein F (OmpF) [3], osmoporin (OmpC) [4], phosphoporin (PhoE protein) [5] and maltoporin (LamB porin) [6].

OmpF porin allows diffusion of hydrophilic, polar molecules with low molecular weight (600-700 Da), including water, ions, antibiotics, glucose, and other nutrients [2]. OmpF is a voltage-dependent ion channel, having a critical voltage (Vc = < 100 mV) that is affected by low pH, pressure, polycations and membrane oligosaccharides and has selectivity for small cations ($Li^+ < Na^+ < K^+$) [7, 8].

It is considered that β-lactam antibiotics enter in the periplasmic space through porins [9]. Has been shown that induced mutations in OmpF residues lead to

conformational modifications which affect the transport of antibiotics through these porins [10, 11]. Ceftazidime, used as an experimental model in this study, is a third generation cephalosporin, resistant to most beta-lactamases and is active on a broad range of Gram-negative and Gram-positive bacteria. The enzymes beta-lactamase, known as penicillinase, act on antibiotics' structure by disrupting the four-atom ring (β-lactam) of antibiotics. In Gram-negative bacteria, these enzymes are released into the environment when antibiotics are present [12].

Planar lipid bilayers technique (BLM – painted, folded and tip-dip methods) represents an important method for studying different single channels reconstituted in artificial lipid bilayers [13]. Therefore, relevant biophysical characteristics (selectivity, pH and voltage dependency) of porins, like OmpF, can be determined using these electrophysiological approaches [14].

The goal of the present study was to characterize the insertion of porin OmpF, purified from E. coli, in phosphatidylcholine lipid bilayers, using tip-dip method, and to identify the electrophysiological features in the presence of ceftazidime, a third-generation cephalosporin antibiotic.

2. MATERIALS, METHODS

Using tip-dip bilayer technique [13], artificial membranes were done using 20 mg/mL lecithin (containing 17% phosphatidylcholine – PC) solubilized in decane. At the solvent/air interface, a monolayer was made and by dipping a borosilicate glass pipette tip (with a resistance of 6–8 MΩ) into the lipid, a monolayer was formed. Immersing a second time the pipette tip, a bilayer with a giga-seal (20-30 GΩ) formation occurs.

Reconstitution of ion channels was performed by adding OmpF at a final concentration of 1 μg/ml. For studying the interaction between OmpF channel and antibiotics, ceftazidime (56.8 μg/ml) was used.

The voltage across the membrane was clamped at different values using a WPC-100 amplifier (ESF electronic, Göttingen, DE), the current was filtered at 1 KHz with a 4-pole Bessel low-pass filter and sampled at 10 KHz with a Digidata 1322A AD/DA interface (Axon Instruments, Sunnyvale, CA). Recordings were analyzed with pClamp 8.1 software (Axon Instruments, USA), Origin 6.0 (Microcal Software Inc., USA). All data were expressed as means ± SEM. Lecithin (17% PC), n-decane, potassium chloride were purchased from Sigma-Aldrich, and E. coli purified OmpF was kindly provided by Professor Mathias Winterhalter.

3. RESULTS, DISCUSSIONS

The increased number of pathogenic bacteria presenting multiple antibiotic resistance mechanisms is becoming an alarming clinical problem. Cephalosporin family compounds belong to the group of the most important antibacterial agents, able to interfere with biosynthesis of peptidoglycans, major components of the bacterial wall. Before reaching the target, antibiotics must overcome the outer membrane barrier.

Taking into account that OmpF represents the main entrance way of these antibiotics, it is important to evaluate the conductance pattern of OmpF in the absence/presence of ceftazidime.

Experiments with reconstituted OmpF porin (1 μg/ml) in tip-dip lipid bilayers, revealed that porin preferentially inserts in PC bilayers in 3 M KCl solutions. Mean single-channel conductance was 129.58 ± 5.4 pS (n = 8) and openings average time 86 ± 10.23 ms (n = 8), for an imposed voltage of - 70 mV.

In figure 1 can be observed single-channel openings with fast kinetics and openings in 3-steps with slow kinetics.

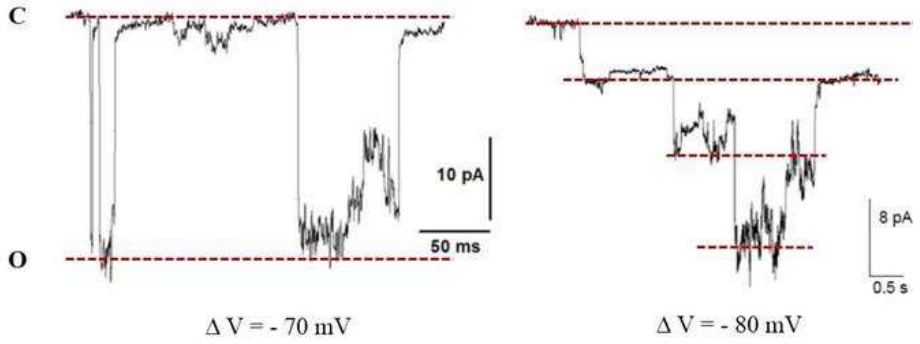

Figure 1. Representative recordings of OmpF channel activity at -70 mV. Mean conductance was 129.58 ± 5.4 pS (n=8) and mean open time 86 ± 10.23 ms (n = 8). At - 80 mV subconductance states of inserted OmpF can be observed. C represents the closing state, O the open state and n, the number of OmpF openings.

At holding potentials of -70 and -80 mV (Figure 1) can be observed subconductance states, behaviour which diminishes at -130 mV (data not shown). The same pattern was obtained in BLM bilayers and in giant liposomes [15]. At potentials of -150 mV the „flickering" effect (noisy recordings) was observed and at > 200 mV a complete destabilization of the lipid bilayer occured.

Experiments conducted in the presence of ceftazidime (53.8 µg/ml) emphasized an increase in OmpF conductance from 129.58 ± 5.4 pS (n = 8) to 171.9 ± 11 pS (n = 9), and a significant increase in the average lifespan from 86 ± 10.23 ms to 2.01 ± 0.5 s (Figure 2).

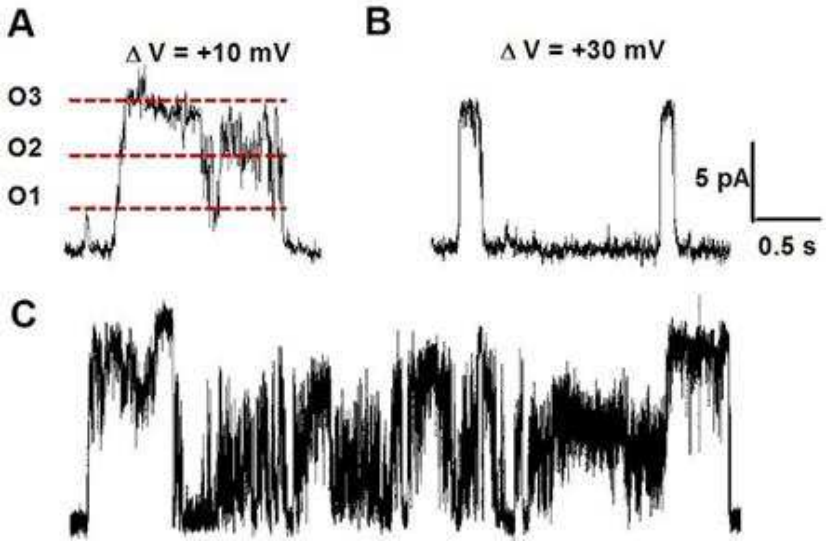

Figure 2. Representative recordings of OmpF channel mediated by ceftazidime (53.8 µg/ml). Recordings were performed at +10 mV (A, C) and +30 mV (B). Unitary mean conductance was 171.9 ± 11 pS (n = 9) and mean open time 2.01 ± 0.5 s (n = 9). O_1, O_2, O_3 represent the subconductance open states and n, the number of OmpF openings.

The interaction of ceftazidime with the OmpF channel can be identified by current fluctuations, easily seen in figure 2 C. Our previous work, done with outer membrane fragments of E. coli no. 2662 strains, have shown that ceftazidime and ciprofloxacin can modulate the ionic current through these porins [16].
Openings with fast kinetics (Figure 2 B) are often associated with monomer activation. For a trimer, the conductance average was 1228 ± 115 pS and for a monomer ~ 409 pS, value similar to that mentioned in literature: ~ 313 pS [16].
The results indicate an increase in conductance and also changes in the mean open lifetimes of OmpF channels in the presence of ceftazidime. Thus, a possible mechanism of translocation through the pore of the antibiotic may occur.
These results are important for improving the pharmacological dosage of ceftazidime administered in the clinic and to reduce the drug resistance of Gram-negative bacteria, in particular of E. coli strains, to cephalosporins.

4. CONCLUSIONS

- ✓ OmpF preferentially inserts in PC bilayers at negative potentials and in 3 M KCl solutions;
- ✓ Ceftazidime increases the conductance and the open lifetime of OmpF channel;
- ✓ The interaction between OmpF reconstituted in artificial lipid bilayers and antibiotics, could generate a quantitative description of these processes that may be useful in selecting new generations of antibiotics and to increase the efficiency of existing ones.

4. PERSPECTIVES

Microarrays are used as screening tools for detecting bacteria and parasites [17]. This approach, combined with molecular biology techniques, provides a rapid and efficient detection of these microorganisms. A variety of biosensors based on antibodies have been tested for pathogen recognition: electrochemical, mass-based, magnetic and optical [18].
Taking into consideration these findings, it can be designed microchips with specific antibodies, for bacteria identification in water samples from different contaminated areas.

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