CHARACTERIZATION AND CHEMICAL MODIFICATION OF SMALL ANION SPECIFIC CHANNELS FORMED IN LIPID BILAYER MEMBRANES BY OUTER MEMBRANE PROTEIN P OF *PSEUDOMONAS AERUGINOSA*

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The movement of small molecules and ions across the outer membrane of gram-negative bacteria is mediated by a class of major proteins named porins (1). The porins form generally large water-filled pores with a diameter of 1.3–2.3 nm in the outer membrane (1) and in lipid bilayer membranes (2). These pores have a defined exclusion limit for hydrophilic solutes (3). A new outer membrane protein has been found to be induced in *P. aeruginosa* grown low-phosphate media. Studies with mutants have suggested that this protein, named protein P, is part of the phosphate uptake system (4). Lipid bilayer experiments suggested that the diameter of the protein-P pore is much smaller than that of the other porins(4).

MATERIALS AND METHODS

Artificial lipid bilayer membranes were obtained from a 1–2 wt/100 solution of diphytanoylphosphatidylcholine (Avanti Polar Lipids, Birmingham, AL) in *n*-decane. The hole used for membrane formation had an area between 0.1 and 0.2 mm^2. The aqueous salt solutions (Merck, Darmstadt, G.F.R.; analytical grade) were used unbuffered with pH ~6. Calomel electrodes with salt bridges were inserted in the aqueous compartments on both sides of the membrane. The membrane current was amplified with a Keithley 425 current amplifier, monitored with a storage oscilloscope, and recorded with a strip chart recorder. Protein P was isolated from outer membranes of *P. aeruginosa* PA01 strain H 103 as described earlier (4). It was used in a concentrated stock solution containing 280 μg/ml porin P, 5 mM Tris-HCl, 0.1% dodecylsulfate and 3 mM azide. Acetylation and succinylation of protein P was performed

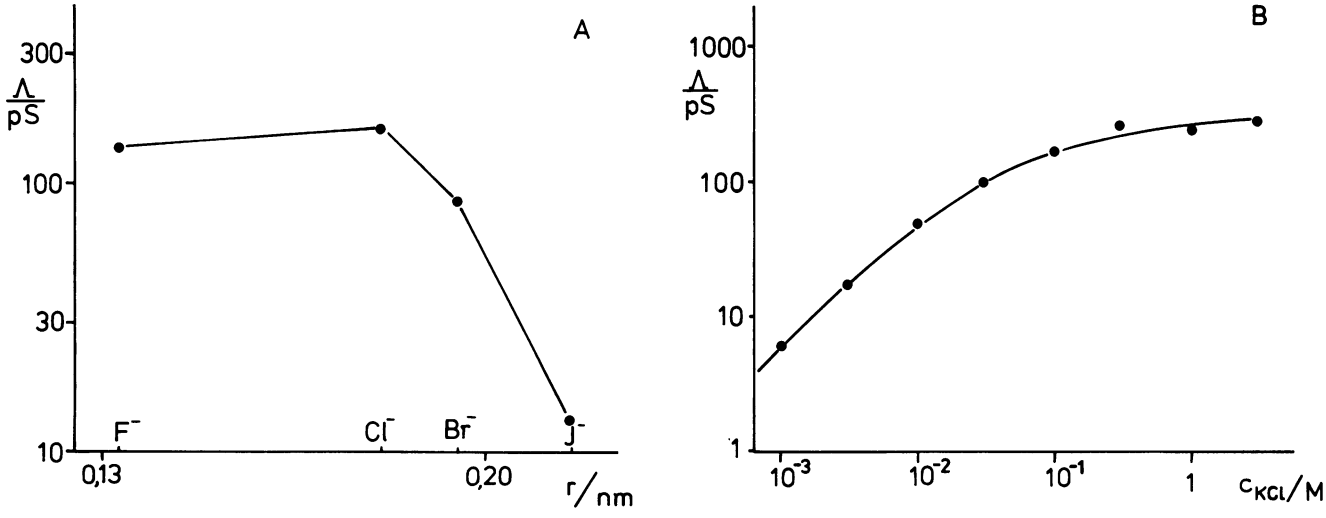

FIGURE 1 *A*, single channel conductance of the P-porin channel as a function of the radius of the anion according to Pauling. The concentration of the potassium salts was 100 mM. V_m = 50 mV; T = 25°C. The aqueous phase contained ~10 μg/ml protein P. *B*, single channel conductance of the P-porin channel as a function of the KCl-concentration in the aqueous phase. V_m = 50 mV; T = 25°C. The protein-P concentration was between 10 μg/ml and 100 μg/ml.

using acetic anhydride or succinic anhydride, as has been published elsewhere (5).

RESULTS AND DISCUSSION

The addition of purified protein P in small amounts (final concentrations ~100 μg/ml) to the aqueous solutions bathing a black lipid bilayer membrane resulted in a conductance increase of many orders of magnitude. The origin for the conductance increase is the formation of pores in the artificial membranes. The current steps were found to be fairly homogeneous compared with those observed with other porins from *P. aeruginosa* (6) or from other gram-negative bacteria (7, 8). The single-channel conductance of the pores formed by protein P was dependent on the type of anion present in the aqueous phase. Fig. 1*A* shows the dependence of the single-channel conductance on the size of the anion. The small increase of the single channel conductance from F^- to Cl^- and the subsequent decrease in the series Cl^-, Br^- and T^- indicates that hydration shell and size of the anions are important factors for the ion permeation through the channel. The protein-P channel is highly specific for anions. This has also been shown by zero-current potential measurements (9).

The dependence of the single channel conductance on the Cl^- concentration is given in Fig. 1 *B*. The single-channel conductance saturated at higher anion concentrations, suggesting single ion occupancy of the channel, i.e., only one binding site inside the channel.

The nature of the selectivity filter was probed by acetylation and succinylation of the amino groups of protein P. This resulted in small changes in the mobility of protein P on dodecylsulfate polyacrylamide gels. It is interesting to note that 100% of the protein was present as trimeric aggregates after chemical modification. Succinylation totally blocked ion movement through the channel. Acetylation caused a reduction of the single channel conductance to <10% of the value for native protein P trimers, as shown in Fig. 2. In addition, the anion selectivity was drastically reduced and saturation of single channel conductance at higher salt concentrations was no longer observed. It is suggested that lysine groups on the mouth and/or on the selectivity filter of the protein-P channel provide the basis for its strong specificity for anions.

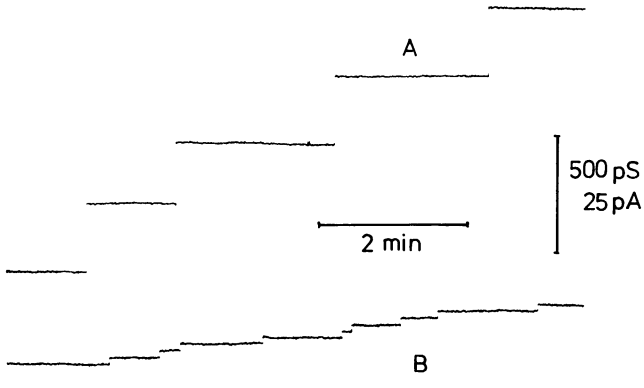

FIGURE 2 Single channel records of current steps in the presence of native protein P (*A*) and acetylated protein P (*B*). The aqueous phase contained 1 M KCl, pH 6, and 10 μg/ml protein P. V_m = 50 mV; T = 25°C.

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