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AN ANION-SELECTIVE CHANNEL FROM THE *PSEUDOMONAS AERUGINOSA* OUTER MEMBRANE

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Protein P from *Pseudomonas aeruginosa* outer membrane was reconstituted in lipid bilayer membranes from diphytanoylphosphatidylcholine. The reconstitution resulted in the formation of anion-selective channels with a conductance of 160 pS for 0.1 M chloride solution. The channels were at least 100-times more selective for anions than for cations as judged from zero-current membrane potentials. The single-channel conductance was dependent on the size of the different anions and saturated at higher salt concentrations suggesting single ion occupancy of the protein P channel.

The permeation of anions through membranes is poorly understood at present and only a few anion-selective transport systems like band 3 from red cells [1] and the chloride channel in *Torpedo* electric organ [2] have been extensively studied. Furthermore, the isolation, purification and successful reconstitution of an intact anion transport has not been demonstrated to date. Here we show the reconstitution of a 48 kDa polypeptide protein P from *Pseudomonas aeruginosa* outer membrane and the formation of an anion-selective channel by this protein. Protein P is presumably organized as trimers of three identical polypeptides (Angus, B.L. and Hancock, R.E.W., unpublished data) and it is very likely involved in the molecular sieving properties of the outer membrane of *P. aeruginosa* which make this organism quite resistant to antibiotics [3,4]. Protein P was first discovered on gels from outer membrane of bacteria living on phosphate minima media [3].

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

Protein P from *Pseudomonas aeruginosa* outer membrane was isolated and purified as published earlier [3]. It was used as a concentrated stock solution (0.53 mg/ml protein P/0.1% sodium dodecyl sulfate/5 mM Tris-HCl (pH 8)/3 mM sodium azide). Black lipid bilayer membranes were obtained from a 1% (w/v) solution of diphytanoylphosphatidylcholine (Avanti, Biochemicals, Birmingham, AL) in *n*-decane according to established procedures [5]. When protein P was added in small quantities (final concentration around 100 ng/ml) to the aqueous solution in which an artificial lipid bilayer membrane was immersed a large increase in membrane conductance of many orders of magnitude was observed. The conductance increase was dependent on the protein concentration in the aqueous phase in an approximately linear manner, if the aqueous solution contained at least 0.1 M salt. At low salt concentration only poor reconstitution was obtained and large concentrations of the porin were needed to obtain one or two orders of conductance increase over ground level conductance of the membranes (10^{-7}–10^{-8} S · cm^2). The origin of the

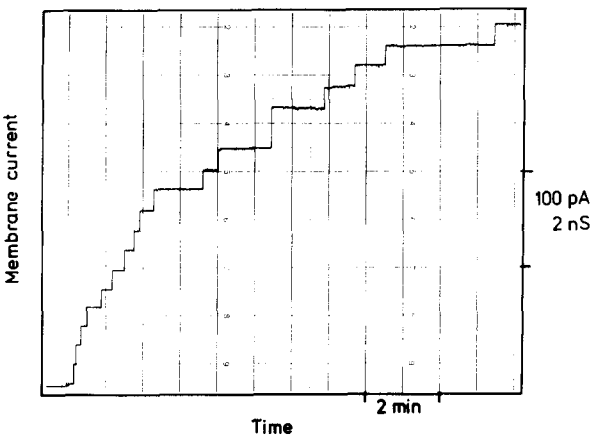

Fig. 1. Single-channel record of a diphytanoylphosphatidylcholine/*n*-decane membrane in the presence of 10 ng/ml protein P and 1 M KF in the aqueous phase. The record was taken from a membrane with a small area (0.2 mm^2) in order to minimize membrane noise. The signal was amplified with Keithley 427 current amplifier and monitored with a strip chart recorder. 50 mV were applied through calomel electrodes with salt bridges; $t = 25°C$; pH 6.

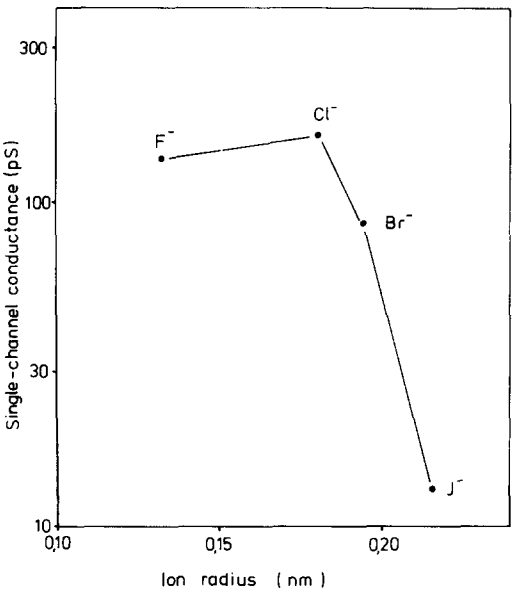

Fig. 2. Single channel conductance of the P-porin channel in diphytanoylphosphatidylcholine/*n*-decane membranes as a function of the ion radius of the anion according to Pauling. The concentration of the potassium salts was 100 mM. The data points represent the means of at least 100 channels. 50 mV were applied to the membranes through calomel electrodes with salt bridges. The aqueous phase contained about 10 ng/ml protein P and the pH was kept at 6; $t = 25°C$.

conductance increase is the formation of pores in lipid bilayer membranes. Fig. 1 shows current steps observed with protein P on a diphytanoylphosphatidylcholine membrane in the presence of 1 M KF. The size of the current fluctuations was found to be fairly homogeneous in contrast to findings with porins from *Salmonella* [6] or *Escherichia coli* [5]. Whereas with these porins at least a factor of two has been found between smallest and largest pore conductances, the single channels found with porin P agreed within 10–20% and a fairly sharp histogram was obtained. Most conductance fluctuations were directed upward and terminating steps were only rarely observed. This indicated that the lifetime of the pores in the lipid bilayer membranes was long and exceeded usually the duration of a single-channel experiment which was on the order of 20 to 30 min. The lifetime of single steps was found to be independent on voltage and a linear current-voltage relationship was observed for the protein P channels up to 200 mV.

Surprisingly the single-channel conductance was not dependent on the type of cation present in the aqueous solution. For 100 mM chloride solution the single-channel conductance was about 160 pS irrespective of size and nature of the cation. A much larger variation of the single-channel conductance Λ was obtained if the anions were varied. Fig. 2 shows the dependence of the single channel conductance Λ of 100 mM salt solutions as a function of the size of the anion. The corresponding cation was in all cases potassium. The conductance appears to be highest for chloride whereas it is lowest for iodide. Besides the halogenides a variety of other anions like nitrate and nitrite are also permeable through the porin P channel whereas in the presence of salts with large organic anions such as $Hepes^-$, no conductance fluctuations could be observed at all.

Large organic anions like $Hepes^-$ and butyrate were found to be impermeable or to be only little permeable (see above) through the porin P channel. The origin of the anion selectivity of the P-porin pore from *Ps. aeruginosa* outer membrane is therefore presumably a selectivity filter containing at least one positive charge. This is also reflected in the concentration dependence of the

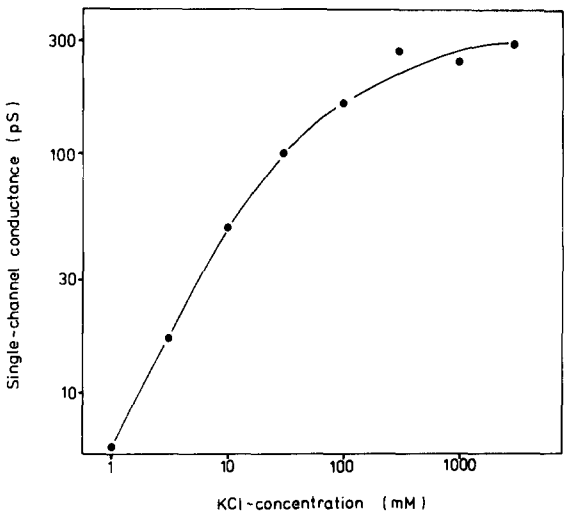

Fig. 3. Single-channel conductance of the P-porin channel as a function of the potassium chloride concentration in the aqueous phase bathing membranes from diphytanoylphosphatidylcholine/*n*-decane. The data points represent the means of at least 100 channels. 50 mV were applied to the membranes. The aqueous phase contained about 10 ng/ml protein P for salt concentrations above 10 mM. For 10 mM and lower concentration 100 ng/ml protein P had to be added in order to receive a sufficient number of single channels; $t = 25°C$, pH 6.

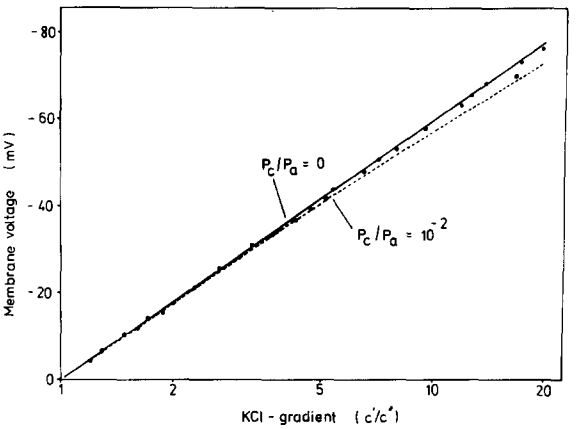

Fig. 4. Zero-current membrane potential V of diphytanoylphosphatidylcholine/*n*-decane membranes in the presence of protein P measured as a function of a KCl-gradient across the membrane. V is defined as the difference of the potential on the dilute side (c') minus the potential on the concentrated side (c''). V was measured with a Keithley 610 C electrometer through calomel electrodes with salt bridges. The lines were drawn according to the Goldman-Hodgkin-Katz equation assuming $P_c/P_a = 10^{-2}$ (broken line) or $P_c/P_a = 0$ (full line). Results of seven different membranes are shown; $t = 25°C$; pH 6.

single-channel conductance. Fig. 3 shows the dependence of the single-channel conductance on the KCl-concentration in the aqueous phase. A linear conductance-concentration relationship was only observed with KCl-concentrations as small as 1 mM and 10 mM. The single-channel conductance approached saturation for higher salt concentrations and no further conductance increase occurred above 0.3 M KCl. The conductance concentration curve given in Fig. 3 could be fitted easily assuming single occupancy of the pore, i.e. one binding site in the pore for anions [6].

The high selectivity of the P-porin for anions has also been shown for multichannel systems measuring zero-current membrane potentials in the presence of a salt gradient across the membrane. Fig. 4 shows experiments of this type. Diphytanoylphosphatidylcholine membranes were formed in 10 mM KCl-solution and protein P was added after blackening of the membranes. After the conductance had increased about 100-fold after that of unmodified membranes, the instrumentation was switched to the measurements of zero-current potential and the concentration on one side of the membrane was raised by adding small aliquots of concentrated KCl-solution. The zero-current membrane potential V being negative on the more dilute side was found to increase with increasing salt gradient across the membrane (Fig. 4). The results could be reasonable well fitted to the Goldman-Hodgkin-Katz equation:

$$V = \frac{RT}{F} \ln \frac{P_c c'' + P_a c'}{P_c c' + P_a c''}$$

(R gas constant, T absolute temperature, F Faraday constant, P_c and P_a permeability ot the cation and the anion, respectively, c' and c'' the salt concentrations on either side of the membrane) assuming either a permeability ratio $P_c/P_a = 0$ (Nernst equation, full line) or $P_c/P_a = 10^{-2}$ (broken line). According to the results for Fig. 4 the permeability of the porin P channel for chloride is at least 100-times higher than that for K^+. This finding is consistent with the results from the single-channel analysis.

Protein P forms presumably trimers of three

identical subunits in *Ps. aeruginosa* outer membrane (Angus, B.L. and Hancock, R.E.W., unpublished data). It is still unclear whether this and the other porin trimers from Gram-negative bacteria contain one or three pores although lipid bilayer experiments favor the existence of only one pore [7–9]. In preliminary experiments we observed blockage of the porin channel by divalent and trivalent anions. Such experiments may help to decide the question if the porin trimers contain one or three pores.

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