AN ANION-SELECTIVE CHANNEL FROM THE PSEUDOMONAS AERUGINOSA OUTER MEMBRANE

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(Received December 8th, 1982)

Key words: Outer membrane; Anion selectivity; Channel formation; Lipid bilayer; Membrane reconstitution; (Ps. aeruginosa)

Protein P from Pseudomonas aeruginosa outer membrane was reconstituted in lipid bilayer membranes from diphyanoylphosphatidylcholine. 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 Protein P is presumably organized as trimers of three identical polypeptides (Angus, B.L. and Hancock, R.E.W., unpublished data) and it is very likely involved in the molecular sieving properties of the outer membrane of P. aeruginosa which make this organism quite resistant to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: Hapes, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid.

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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 Δ was obtained if the anions were varied. Fig. 2 shows the dependence of the single channel conductance Δ 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
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:

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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 of 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.

This work was supported by grants of the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 138 and Be 865/1-1) and by grants to R.E.W. H. from the National Scientific and Engineering Research Council of Canada and the Canadian Cystic Fibrosis Foundation.

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