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SINGLE-CHANNEL PARAMETERS OF GRAMICIDIN A, B AND C

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SUMMARY

The single-channel conductance Λ and the mean channel lifetime τ^* of natural and synthetic gramicidins A, B, and C has been studied Significant differences in Λ were found between gramicidin A and B, both gramicidins differ only in one amino acid (tryptophan replaced by phenylalinine) The distribution of Λ is narrow in glycerylmonooleate membranes but considerably broader in dioleoyl phosphatidylcholine and dioleoyl phosphatidylethanolamine membranes The ratio of the single-channel conductances in glycerylmonooleate and dioleoyl phosphatidylcholine membranes is only about two and is considerable smaller than the conductance ratio of nonactin-mediated cation transport This finding suggests that dipolar potentials at the membrane/solution interface have little influence on the conductance of the gramicidin channel

INTRODUCTION

The mechanism of ion transport in hydrophilic pores may be studied using model compounds of known chemical structure Such compounds are gramicidins A, B and C, linear peptides consisting of 15 apolar amino acids (Fig 1) In a lipid bilayer membrane the gramicidins form pore-like channels that are permeable to small

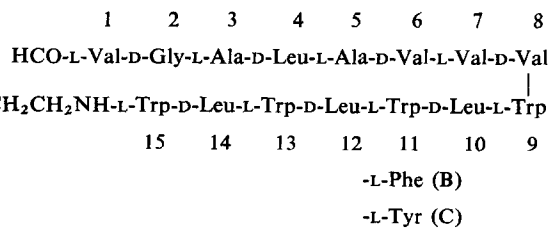

Fig 1 Structure of valine gramicidin A Valine gramicidins B and C differ from valine gramicidin A in that L-tryptophan in position 11 is replaced by L-phenylalanine and L-tyrosine, respectively

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univalent cations (for a survey of the literature, see refs 1–3) The available experimental evidence favours the view that the channel consists of a helical dimer in which the peptide carbonyl groups line the central hole along the axis, whereas the hydrophobic residues lie on the exterior surface of the helix [4–8]

An important question concerns the relationship between chemical structure and transport properties of ion channels This problem may be attacked by studying chemical analogues of the channel-forming molecule In the following we report the results of studies of the single-channel properties of gramicidins A, B and C, which have been synthetized or isolated from the natural gramicidin mixture The structure of gramicidin A is shown in Fig 1, gramicidins B and C differ from A in that L-tryptophan in position 11 is replaced by L-phenylalanine and L-tyrosine, respectively Most of the previous studies have been carried out with the natural gramicidin mixture which contains about 72 % A, 9 % B and 19 % C [9] We find that the replacement of a single amino acid in the pentadecapeptide may result in a significant change of the single-channel conductance Furthermore, characteristic differences of the single-channel parameters in different lipids are observed

MATERIALS AND METHODS

Natural gramicidin A, B and C were separated by countercurrent distribution in the solvent system chloroform/benzene/methanol/water = 15 15 23 7 (by vol) [10] Natural gramicidin A consisted of a mixture of the valine and the isoleucin analogue (valine in position 1 replaced by isoleucine) Natural gramicidins B and C were pooled fractions from the countercurrent distribution consisting of the valine analogues only

Valine gramicidins B and C were synthetized on 1 % cross-linked polystyrene resins and purified by countercurrent distribution in the solvent system given above [11]

Glycerylmonooleate was obtained from Nu Chek Prep, Elysian, Minnesota (U S A) Dioleoyl phosphatidylcholine and dioleoyl phosphatidylethanolamine were synthetized by K Janko [13, 15] The purity of these lipids was checked by thin-layer chromatography Optically black lipid films were formed as described previously [1, 12] in a thermostatted Teflon cell from 1–2 % lipid solutions in *n*-decane (T = 25 °C) The area of the film was about $4 \cdot 10^{-4}$ cm^2 The concentration of the electrolyte (different alkali chlorides) in the aqueous solutions was 1 M, and the pH was between 6 0 and 6 5 Gramicidin was added from a methanolic stock solution to the aqueous phase The experimental set-up for the measurement of conductance fluctuations arising from the formation and the disappearance of single channels has been described previously [1] The measurement was started 20–30 min after the membrane had turned completely black After this time the electrical capacity was nearly stationary, the capacity values were 0 39 μF/cm^2 (glycerylmonooleate), 0 37 μF/cm^2 (dioleoyl phosphatidylcholine) and 0 38 μF/cm^2 (dioleoyl phosphatidylethanolamine) [14, 15] The fluctuating current at a voltage of 100 mV was recorded on magnetic tape and the signal subsequently transferred on an expanded time-scale to a strip-chart recorder

RESULTS AND DISCUSSION

For each system studied a histogram was constructed from the observed values of the single-channel conductance Λ Two examples are given in Fig 2 The conductance values Λ at the peak of the probability distribution are listed in Tables I and II for the different systems Our Λ values of gramicidin A in glycerylmonooleate membranes are in agreement with those of Hladky and Haydon [5] who obtained $\Lambda_K = 4\,2\ \ 10^{-11}\ \Omega^{-1}$ in 1 M KCl at 23 °C (Our value, corrected for this temperature [1] would be $\Lambda_K = 4\,6\ \ 10^{-11}\ \Omega^{-1}$) It is seen from Table I that natural and synthetic gramicidin B closely agree in their conductance values, the same being true for natural and synthetic gramicidin C

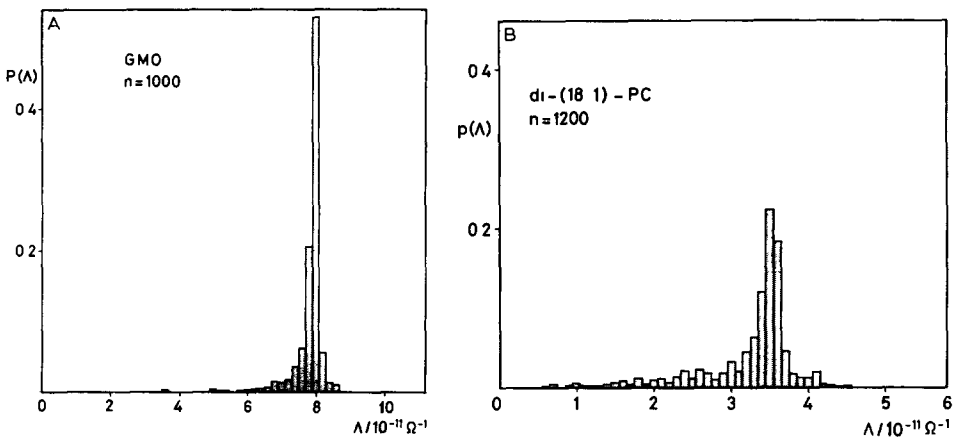

Fig 2 Probability $p(\Lambda)$ of the occurrence of a conductance fluctuation of magnitude Λ $p(\Lambda)$ is the number of events within an interval of width $\Delta\Lambda = \pm 2\ \ 10^{-13}\Omega^{-1}$ centered at Λ, divided by the total number n of events Natural gramicidin B, 1 M CsCl, 25 °C Di-(18 1)-PC, dioleoylphosphatidylcholine, GMO, glycerylmonooleate

Gramicidin B in which tryptophan in position 11 is replaced by the less polar amino acid phenylalanine is found to have a smaller Λ than gramicidin A for all ions studied (Table I) The difference between gramicidin C, (tryptophan replaced by the more polar tyrosine) and gramicidin A is insignificant Interestingly, not only the absolute conductance values are different for gramicidin A and B, but also the conductance ratio Λ_{Cs}/Λ_K This means that the ion specificity of the channel changes slightly from A to B

In general, the single-channel conductance Λ depends both on the probability that an ion enters the channel from the aqueous phase and on the ion mobility in the channel [16] The two parameters may be separated, in principle, if Λ is measured at ion concentrations sufficiently high to reach saturation levels of the channel conductance [5] If it is assumed that the saturation values of Λ for the different ion species correspond to the same occupancy of the channel, then the extrapolated saturation values reflect differences in ion mobility In view of the difficulties in the determination of activity coefficients of single ion species we have not carried out such an analysis, but have restricted ourselves to electrolyte concentrations of 1 M where the channel is

TABLE I

Single-channel conductance λ and mean channel lifetime τ^* of different natural and synthetic gramicidins in glycerylmonooleate membranes 25 °C, 100 mV, electrolyte concentration 1 M. λ is the conductance value at the peak of the probability distribution (Fig 2). $(\Delta\lambda)_{75}$ is the interval around λ covering 75 % of all observed events. τ^* was determined from the slope of the plot of log N versus τ, where N is the number of events which had a lifetime longer than τ [5]. In each case at least $n = 800$ events have been used for the evaluation of λ and τ^*.

Gramicidin	Ion	λ ($10^{-11}\Omega^{-1}$)	λ_i/λ_{K^+}	$(\Delta\lambda/\lambda)_{75}$	τ^* (s)
A, natural	Na^+	2.5	0.50	0.09	0.21
	K^+	5.0	1.00	0.06	0.22
	Cs^+	9.0	1.80	0.04	0.24
B, natural	Li^+	0.42	0.12	–	–
	Na^+	1.74	0.50	0.04	0.22
	K^+	3.5	1.00	0.09	0.20
	Cs^+	8.0	2.29	0.03	0.29
B, synthetic	Na^+	1.76	0.50	0.04	0.26
	K^+	3.5	1.00	0.09	0.19
	Cs^+	7.9	2.26	0.03	0.21
C, natural	Li^+	0.56	0.12	–	–
	Na^+	2.5	0.52	0.07	0.17
	K^+	4.8	1.00	0.06	0.21
	Cs^+	9.2	1.92	0.07	0.26
C, synthetic	Li^+	0.50	0.11		0.25
	Na^+	2.4	0.51	0.17	0.20
	K^+	4.7	1.00	0.19	0.21
	Cs^+	9.2	1.96	0.11	0.21

TABLE II

Single channel conductance λ and mean channel lifetime τ^* of natural and synthetic gramicidin B in different lipids. 25 °C, 100 mV, electrolyte concentration 1 M.

Lipid	Ion	λ ($10^{-11}\Omega^{-1}$)	λ_i/λ_{K^+}	$(\Delta\lambda/\lambda)_{75}$	τ^* (s)
Natural gramicidin B					
Glyceryl monooleate	Li^+	0.42	0.12	–	–
	Na^+	1.74	0.50	0.04	0.22
	K^+	3.5	1.00	0.09	0.20
	Cs^+	8.0	2.29	0.03	0.29
Dioleoyl phosphatidyl-ethanolamine	Na^+	0.95	0.59	0.16	–
	K^+	1.6	1.00	0.07	0.35
	Cs^+	5.2	3.25	0.15	0.50
Dioleoyl phosphatidyl-choline	K^+	1.8	1.00	0.43	0.57
	Cs^+	3.5	1.94	0.23	0.54
Synthetic gramicidin B					
Glyceryl-monooleate	Na^+	1.76	0.50	0.04	0.26
	K^+	3.5	1.00	0.09	0.19
	Cs^+	7.9	2.26	0.03	0.21
Dioleoyl phosphatidyl-ethanolamine	K^+	1.8	1.00	0.11	–
	Cs^+	5.9	3.28	0.23	–

sufficiently far from saturation [5]

Table II shows the single-channel parameters of gramicidin B in membranes made from different lipids The comparison of Λ in glycerylmonooleate and phosphatidylcholine is particularly interesting, since studies of the nonactin-mediated K^+ transport gave evidence for considerable differences in interfacial potential of membranes made from the two lipids The interior of a glycerylmonooleate membrane was estimated to be less positive by 120–130 mV than the interior of a phosphatidylcholine membrane [17] As may be seen from Table II, the difference in Λ in membranes made of these two lipids is in the right direction, but the single-channel conductance in a glycerylmonooleate membrane is greater by a factor of only about two whereas the conductance ratio in carrier-mediated cation transport was of the order of 100 Accepting a potential difference at the interface of 120–130 mM, this indicates that the electrostatic energy of an ion inside the channel is little affected by the interfacial potential of the membrane In the absence of a more comprehensive theory of the electrostatics of ion channels [18], a detailed discussion of the above findings seems premature

As illustrated in Fig 2, the probability distribution $p(\Lambda)$ for the occurrence of a given value of Λ is relatively narrow in the case of a glycerylmonooleate membrane, but considerable broader for phosphatidylcholine membranes As an approximate measure of the width of the distribution we have introduced the interval $\Delta\Lambda$ covering 75 % of the recorded events The ratios $\Delta\Lambda/\Lambda$ are listed in Tables I and II for the different systems For glycerylmonooleate membranes $\Delta\Lambda/\Lambda$ is between 0 03 and 0 19, whereas considerably larger values (0 23 and 0 43) are found for phosphatidylcholine membranes Synthetic gramicidin C tends to give a somewhat broader distribution than natural gramicidin C, possibly an indication for the presence of small amounts of chemical analogues in the synthetic product

Of particular interest is the observation that in the case of phosphatidylcholine and phosphatidylethanolamine, where the single-channel conductances Λ were distributed over a considerable range, fluctuations of Λ never occurred within the lifetime of a single channel (this is also apparent from the current records in previous papers [1, 5]) In other words, a newly formed channel always starts with a fixed conductance Λ which stays constant throughout its lifetime τ The next channel may show a significantly different Λ which again remains constant during τ

The fact that Λ is distributed over a considerable range in certain lipids may have a number of different explanations A first possibility would be that all the gramicidins used are still chemically heterogeneous We consider this explanation unlikely because it would imply that the heterogeneity is expressed differently (in terms of Λ) in different lipids Second, there may not be a single form of the channel, but a range of different hydrogen-bonded structures, and one of these structures may be "frozen in" when the channel forms Still another possibility which cannot be excluded at present is that channels form in different lipid environments If this explanation is correct, the patches of constant lipid environment must be rather large This follows from the long lifetime of the channel ($\tau^* \approx 0\,2$ s, see Tables I and II) which, together with an estimated diffusion coefficient of the channel of 10^{-9}–10^{-8} $cm^2\ s^{-1}$ [6], gives a mean diffusion length of several thousand angstroms

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