Biophysical Chemistry 24 (1986) 143–148
Elsevier

BPC 01062

ANALYSIS OF THE ION TRANSFER THROUGH THE CHANNEL OF 9,11,13,15-PHENYLALANYLGRAMICIDIN A

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Received 21st January 1986
Revised manuscript received 2nd April 1986
Accepted 10th April 1986

Key words: Ion transport; Ion channel; 9,11,13,15-Tetraphenylalanylgramicidin A

The behaviour of an analogue of gramicidin A in which all four tryptophanyl residues are substituted by phenylalanyl and which shows a strong voltage effect on the single channel conductance is analyzed on the basis of a 'three-barrier–two-site' model. It is shown that in the gramicidin family the side chains of some amino acids, in spite of their location, which point outside the channel can play a major role in the binding of ions in the channel and thus can significantly modify the energy profile of the channel.

1. Introduction

It is known that the single channel conductance of linear gramicidins [1]

HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-
1 2 3 4 5 6
L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-
7 8 9 10 11 12 13
D-Leu-L-Trp-NH-C_2H_4-OH
14 15

(gramicidin A) depends on the chemical nature of some residues. This was first observed on natural analogues of gramicidin A, namely gramicidin B and C in which residue 11 is Phe and Tyr, respectively [2], and later on chemically N-terminal modified gramicidins [3,4] and was attributed to possible electrostatic interactions between the dipoles of the side chains and the ions in the channel as no major conformational changes of the polypeptide backbone could be detected. That the side chains play a major role in ion movements in the gramicidin pores was suggested for gramicidin A [5,6] and was confirmed by the study of a fully synthetic analogue, called gramicidin M [7], in which all four tryptophanyl residues are substituted by phenylalanine. Indeed, gramicidin M shows a strong voltage dependence of the single channel conductance [8], while those of gramicidin A are, under identical conditions, almost independent of the voltage [2,9–12]. This makes gramicidin M a very attractive model for the understanding of ion movements through transmembrane pores. We report here more detailed investigations on the transfer of Cs^+ through black lipid membranes mediated by this molecule and an analysis of the profile of the ionic channel. This latter point will be discussed in terms of the influence of the side chains on the parameters characterizing the process of the ion movement inside the channel.

2. Materials and methods

Gramicidin M (actually, owing to the availability of some amino acid derivatives, it was chosen

to prepare the enantiomer, i.e., gramicidin M^-) had the same origin as that previously used [8]. The use of the enantiomer cannot affect the conductance properties since black lipid membranes are formed from a racemic lipid (2% glyceryl monooleate from Sigma in decane).

Gramicidin A (actually a mixture of gramicidin A, B and C) (Sigma) was recrystallized from ethanol before use.

Single channel experiments were performed using the same procedure as already described [13].

Voltage jump measurements were carried out using a Philips PM 5715 pulse generator, a Keithley 427 current to voltage convertor and a Tektronix 5441 storage oscilloscope.

All the experiments were done on symmetrical systems.

3. Results and discussion

3.1. General considerations

Before analyzing the electrochemical data obtained on gramicidin M^-, it is worthwhile to recall that, although the conformation of the active form of gramicidin A is still disputed [14,15], it will be shown that from a conformational point of view, both gramicidin A and M^- behave very similarly [16]. Indeed, when observed under identical conditions, both molecules, which differ only in the substitution of the four Trp residues by Phe, adopt the same conformation and undergo very similar time- or concentration-induced transconformations. Therefore, the analysis of the results obtained on the synthetic molecule presented here will be made on the same basis as gramicidin A using the simple 'three-barrier–two-site' (3B2S) model [10,17–19] although another model with three barriers and four sites (3B4S) has been proposed [20,21].

3.2. Voltage dependence of the instantaneous current in multi-channel experiments

Fig. 1 shows the intensity vs. time (I-t) current responses of voltage jump experiments obtained at various CsCl concentrations on highly doped membranes. The behaviour of gramicidin A (right-hand part) under identical conditions is given for comparison purposes.

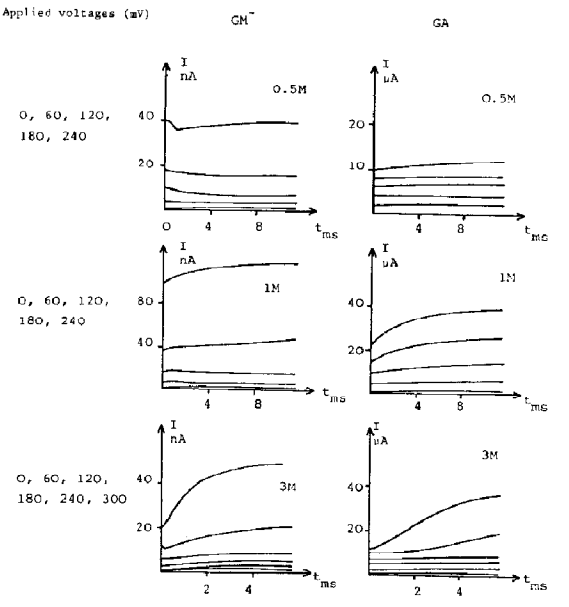

Fig. 1. Drawing of the voltage jump-current response traces obtained on gramicidin M^- (left) and gramicidin A (right) at various applied potentials and CsCl concentrations and at identical bulk concentrations of gramicidin. Increasing applied voltages correspond to increasing transmembrane currents.

The first transient decrease of the current (not shown in the figure) corresponds, as is usual for this kind of experiment, to the charging of the membrane capacitance with a time constant of 10 μs^{-1} for the experimental conditions used here. The second transient which is recorded in the millisecond time range is due to several processes: (i) redistribution of cations between the binding sites of the channels up to the steady state; (ii) variation of the current due to electrostriction phenomena [10,22] or to voltage-induced increase in the number of open channels [23]. However, it is usually admitted that, for voltage jump measurements, at the beginning of the second transient of the I-t response the membrane remains in the initial state, i.e., the occupancy states at the end of the charging step are the same as those at equilibrium. Therefore, the instantaneous trans-

membrane current I_o can be used to describe the system. Note that the origin of the current decline observed for gramicidin M^- at 0.5 M CsCl which also occurs at lower salt concentrations (not shown) is still unexplained. In fig. 2 we have reported the variation of log I_o as a function of the applied voltage. This figure shows, in the 100–300 mV range, a linear behaviour of the log I_o-V variations which is indicative of a single exponential variation of I_o. It can thus be concluded that only one process is rate determining. Further, from the slopes of the log I_o-V variations its corresponding voltage dependence coefficient or electrical distance is 0.33–0.40 (see fig. 2). By analogy with gramicidin A for which the ion-binding sites are located 2.5 Å from the mouths of the channel [24–26], the electrical distance obtained for gramicidin M^- is associated with the translocation process which is the rate-determining step.

3.3. Single channel experiments

Fig. 3 shows the variation of single channel conductances of gramicidin M^- as a function of the applied voltage at various CsCl concentrations. The general trend of the conductance vs applied voltage (Λ-V) curves is the same as that previously reported [8,27]. Note that, in the results reported here, a more accurate exploration in the low-voltage region allows the determination of a non-zero value of the limiting conductances. The variation of these limiting conductances as a function of the Cs^+ concentration is reported in fig. 4. The equilibrium constant of the binding process, K_B, has been estimated from fig. 4 using the procedure reported by Finkelstein and Andersen [28]. Its value (0.93 M^{-1}) deduced from the slope at the origin and the conductance at the plateau is considerably lower than that obtained for gramicidin A (about 2–3 orders of magnitude) [29]. Therefore, it can be concluded that the substitution of the four tryptophanyl residues of gramicidin A by phenylalanyl leads to drastic modifications of the ion-binding process, at least for Cs^+.

Analyses of the Λ-V curves reported in fig. 3 were made as already described [18] assuming an exponential potential dependence of the rate constants $k = k^\circ \exp(\alpha U)$ where α is the voltage dependence coefficient [29]. Under these conditions, for the channel in a mono-occupancy state,

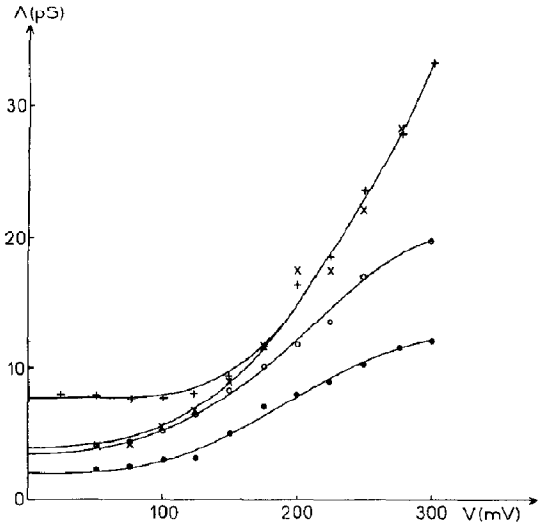

Fig. 3. Variations of the single channel conductances as a function of the applied voltage at various CsCl concentrations: (+) 6 M, (×) 3 M, (○) 1 M, (●) 0.5 M.

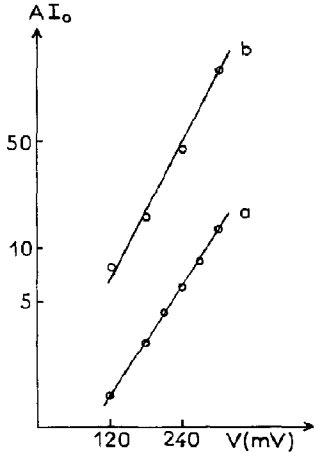

Fig. 2. Variations of I_o (logarithmic scale) as a function of the applied potential. A is an arbitrary coefficient used for normalization. (a) 3 M CsCl, $\alpha_T = 0.33$; (b) 1 M CsCl, $\alpha_T = 0.40$.

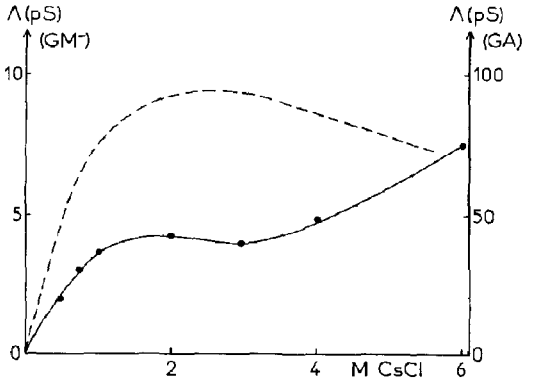

Fig. 4. Variation of the limiting conductances with CsCl concentrations. (———) gramicidin M^-, (- - - - -) gramicidin A [9].

according to the scheme.

$$Cs^+ + GM^- \underset{k''_B}{\overset{k'_B}{\rightleftharpoons}} \underset{B}{Cs^+GM^-} \underset{k''_T}{\overset{k'_T}{\rightleftharpoons}} \underset{C}{GM^-Cs^+} \underset{k''_E}{\overset{k'_E}{\rightleftharpoons}} \underset{A}{GM^- + Cs^+}$$

the channel conductance is

$$\Lambda = e/Vk_T^\circ\left(\left(P_B \exp(\alpha_T U - P_C \exp(-\alpha_T U)\right)\right)$$

On the basis of the voltage dependence coefficient determined above ($\alpha_T = 0.33$–0.40) and of the binding constant $K_B = k_B^\circ/k_E^\circ = 0.93\ M^{-1}$ (thus $k_B^\circ \simeq k_E^\circ$), the single channel conductances have been calculated for different Cs^+ concentrations assuming, as suggested above, that the rate-determining step is the translocation process. The initial values of the various parameters (α_B, α_T, α_E, k_B°, k_T° and k_E°) were chosen as follows: $\alpha_T = 0.38$ (see above) while α_B and α_E were taken to be close to the values of gramicidin A (0.1). Concerning k_B°, k_T° and k_E° as the chemical compositions of the central part of both gramicidins are identical in Urry's model [14] an attempt to fit the Λ-V curves was made using the translocation constant reported for gramicidin A [29]. Therefore, owing to the rate-determining step which is the translocation process, we chose as initial values for k_B° and k_E° higher values than those reported for gramicidin A. Under these conditions, the Λ-V curve fitting failed and could be obtained only be lowering k_T° to $\simeq 10^6\ s^{-1}$ while k_B° and k_E° were kept at $\simeq 10^8$ (see table 1). Note that a deviation of 10% in the parameters given in table 1 is possible and falls within the precision of the experimental data. It must also be mentioned here that fit holds true only for Cs^+ concentrations lower than 3 M. Fig. 5 shows the fit obtained at two Cs^+ concentrations (0.5 and 1 M, corresponding to Cs^+ activities of 0.275 and 0.55, respectively) together with the results obtained at 3 M Cs^+. Increasing Cs^+ concentration leads to an increase in the discrepancy between calculated and experimental Λ-V curves. The latter behaviour has been attributed to a modification of

Table 1

Data of the parameters of the gramicidin M^- channel

$k_B^\circ\ (s^{-1})$	$k_T^\circ\ (s^{-1})$	$k_E^\circ\ (s^{-1})$	α_B	α_T	α_E
1.1×10^8	2.0×10^6	1.18×10^8	0.11	0.38	0.01

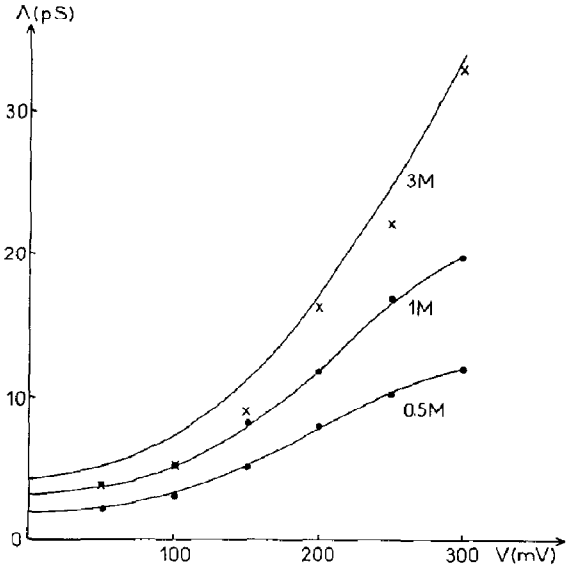

Fig. 5. Variations of the single channel conductances as a function of the applied voltage at various CsCl concentrations (experimental points) compared with the calculated ones (curves) on the basis of the coefficients reported in table 1.

the loading state of the channel (more than one ion).

Examination of table 1 calls for the following comment. As suggested by the multi-channel experiments the fitted data indicate that the translocation is the rate-determining step (no fit could be obtained for the reverse situations, i.e., binding and/or exit steps are rate determining) and the energy barriers calculated from the rate constants are 6.32, 6.28 and 8.65 kcal for the binding, exit and translocation processes, respectively.

4. Conclusion

In conclusion, the ion transport properties of gramicidin M^- can be analyzed on the basis of a three-barrier–two-site model and the main difference with gramicidin A is found in the binding step. For gramicidin M^- the entry barrier and/or the binding well are lowered as compared to gramicidin A. This demonstrates the influence of the side chains, since the only difference between both molecules consists of the substitution of the four tryptophanyl residues by phenylalanyl. Further, as on the basis of a head-to-head dimer model [14,30], these residues are located near the mouth of the channel and thus near the binding sites, it could be expected that the difference in behaviour may have originated mainly from the binding process. As for the role of the side chains, an attempt at an explanation is suggested by the recent findings of Nabedryk and Breton [31] who showed that, for gramicidin A incorporated into dried vesicles, the tryptophanyl side-chains are oriented nearly parallel to the helix surface, allowing them to interact with the backbone peptide groups and thus can modify the orientations of the latters. Thus far, nothing is known about the orientation of the phenyl groups of gramicidin M^-. However, besides a possible effect on the dipole moment, it also seems plausible to suppose that they interact with the peptide groups as well but in a different manner than the indole groups, leading thus to slight modifications of the 'walls' of the channel formed by a π_{DL} helix and will therefore modify the ion-ligand interactions [32]. These conclusions are supported by conformational investigations [16] which indicate that both molecules adopt identical conformations in solution and therefore have very probably the same conformation in their active form.

Acknowledgement

This work was supported by RCP 80605 from the C.N.R.S.

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