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Formation of membrane channels by chrysospermins, new peptaibol antibiotics

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Abstract

The four homologous chrysospermins (Ia–d) are new 19 amino acid peptaibols which form cation selective ion channels in artificial lipid bilayer membranes. Conductance of channels formed by chrysospermins B (Ib) and D (Id) was twice as high (640 pS in 100 mM KCl) as found with chrysospermins A (Ia) and C (Ic). Single channel current traces were recorded for each of the four peptides even at very low (even zero) membrane voltages suggesting that non-gated channels are formed.

Keywords: Peptaibol; Chrysospermin; Lipid bilayer; Channel formation; Voltage-dependent conductance

1. Introduction

Artificial lipid bilayer membranes have widely been used to characterize interactions of drugs with cellular membranes [1–3]. A series of natural products of microbial origin such as linear and cyclic peptides, polyethers and polyene macrolides has been shown to improve ion penetration through biological membranes via formation of pores or carriers, thus enabling passive ion fluxes from inside to outside of cells and vice versa (c.f. Ref. [2]). Amongst the pore-forming agents the so-called peptaibols display particularly effective structures due to their helical peptide backbone composed mainly from hydrophobic amino acids such as α-aminobutyric acid, isoleucine, leucine and valine. Moreover even some hydrophilic constituents such as glutamic acid, glutamine, alanine and proline could to be included [4–11]. Usually, the nitrogen terminus of the 12 to 20 amino acid peptide chain is acylated while the carbon terminal end is reduced to the pertinent amino acid alcohol.

Though the peptaibol family of antibiotics comprises more than 30 different members [4–11] biophysical measurements on model membranes have been carried out so far by use of few representatives of these agents such as alamethicin F-30 [8–11] (II, Fig. 1), suzukacillin, trichorzianin (trichorzianin TA IIIc (III); Fig. 1), trichopolyn-I, hypelcin-A and zervamicin-IIB [7–15]. As a major characteristics of the membrane interactions of alamethicin (II), the formation of ion-penetrable channels was established. These are built up in a time-dependent manner due to the intermolecular association of 5 to 12 alamethicin molecules within the bilayer to ensure the short-term existence of a membrane pore. However, in the case of alamethicin an external voltage applied across the membrane has been necessary to enable an α-helical conformational arrangement of the dipolar peptide molecule that provokes the intermolecular associations and the flip-flop gating mechanism of channel formation [7–11]. With zervamicin-IIB and trichorzianin IIIc the role of the polar carbon end group in ion conveyance has previously been investigated [14,15].

Recently we isolated the chrysospermins (Fig. 1) as new representatives of the peptaibol family of antibiotics from the mycelium of the fungus *Apiocrea chrysosperma* [16,17]. The chrysospermins are composed of a linear peptide chain of 19 amino acids differing in the homologues A, B, C, and D (Ia–d) only by the subunits (isovaline or α-aminoisobutyric acid) at positions 5 and 15. As compared with the well known alamethicin F-60

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Ia AcPhe-Aib-Ser-Aib-Iva-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Aib-Aib-Aib-Gln-Trpol

Ib AcPhe-Aib-Ser-Aib-Aib-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Iva-Aib-Aib-Gln-Trpol

Ic AcPhe-Aib-Ser-Aib-Aib-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Aib-Aib-Aib-Gln-Trpol

Id AcPhe-Aib-Ser-Aib-Iva-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib- Pro-Iva-Aib-Aib-Gln-Trpol

II AcAib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol

III AcAib-Ala-Ala-Aib-Aib-Gln-Aib-Aib-Aib-Ser-Leu-Aib-Pro-Val-Aib-Ile-Gln-Glu-Trpol

Fig. 1. Chemical structures of chrysospermins A (Ia), B (Ib), C (Ic), D (Id), alamethicin (II) and trichorzianin TA IIIc (III). Abbreviations: Gln, glutamine; Trpol, tryptophanol; AcPhe, *N*-acetylphenylalanine.

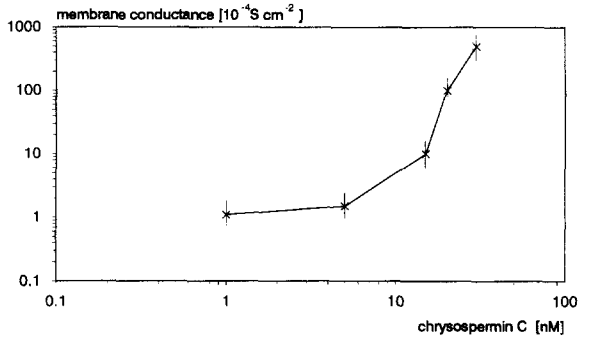

Fig. 2. Membrane conductance versus concentration of chrysospermin C (Ic). Conditions: 20 mM NaCl, pH 7.2; at both sides of the membrane. Ic dissolved in methanol was given to the outer compartment; membrane voltage was 80 mV, negative in the outer compartment.

(II), the chrysospermins are distinguishable by the nature of the amino acids and the absence of free carboxylic groups within the peptide chain. But similar to the trichorzianins (III) the C-terminus is formed by tryptophanol. The interesting antibacterial, antifungal and morphogenetic activities of these new peptaibols (c.f. Refs. [16,17]) attracted us to study the membrane interactions of the chrysospermins A–D (Ia–d) by the use of an artificial model membrane.

2. Materials and methods

2.1. Materials

Chrysospermins were isolated as pure components A (Ia), B (Ib), C (Ic), and D (Id), from the mycelium of *Apiocrea chrysosperma* 634-2 by chromatographic methods including HPLC separation of the four single components of the chrysospermin complex [16,17].

The other chemicals used in the experiments were analytical grade commercial products.

2.2. Measurements employing artificial membranes

Artificial membranes were prepared from soya bean phosphatidylcholine (Sigma; 20 mg in 1 ml n-heptane). Measurement of current by the voltage clamp method was carried out as previously described [18–21]. The measuring cell (12 ml) was equipped with a teflon cylinder (1.2 ml) which contained a hole of 0.5 mm diameter to harbour the black lipid membrane. Both the measuring cell and the inner side of the teflon cylinder were filled with a solution of potassium or sodium chloride ranging from 10 to 100 mM depending on the type of experiment. The measuring device consisted of an operational amplifier model Keithley-301 (USA). Noise-current peak to peak was less than 10^{-13} A in the frequency range 0.1 to 10 Hz. The output voltage was fed to a X–Y voltage plotter. All measurements were carried out at room temperature. The formation of the black lipid bilayer was controlled by the use of a binocular microscope.

3. Results

As shown in Fig. 2 for chrysospermin C (Ic), the chrysospermins (1 to 100 nM) increase the current through an artificial bilayer membrane formed by soya bean phosphatidylcholine if they have been added solely to the outer compartment. This membrane activity appears as similar as it has been reported for alamethicin (II), a typical representative of the peptaibol-type family of peptide antibiotics [1,6–11].

Measurement of the current–voltage curve (Fig. 3), for 0.01 μM chrysospermin C showed that the membrane conductance is weakly voltage-dependent.

Numerical values of the parameters (α) and (m) describing the voltage and concentration dependence of the chrysospermin-induced conductance can be estimated according to Eq. (1):

$$g = g_0[c]^m \exp(\alpha eV/kT) \quad (1)$$

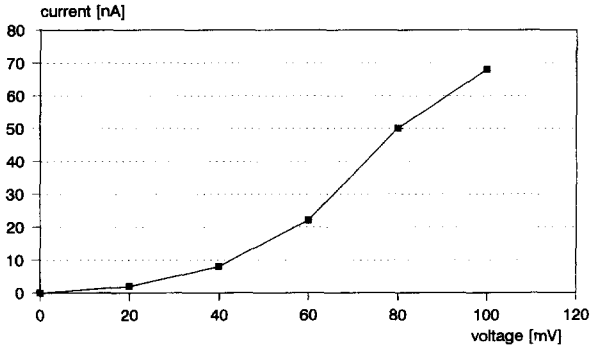

Fig. 3. Current–voltage characteristics of the bilayer membrane in presence of a 0.01 μM concentration of chrysospermin C (Ic). Potassium chloride concentration was 100 mM. The membrane area was approx. 0.1 mm^2.

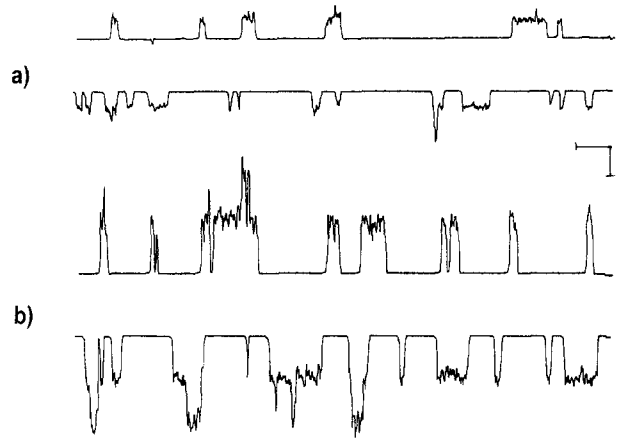

Fig. 4. Comparison of representative single-channel traces chrysospermins C (Ic) D (Id). (a) Chrysospermin D channels. Upper trace: applied voltage −24 mV and symmetrical 100 mM KCl concentrations in the outer and inner compartment. Lower trace: chrysospermin D channels at zero membrane voltage and gradient of KCl (300 mM in the outer and 100 mM in the inner compartment). (b) Chrysospermin C channels. Upper trace: applied voltage −24 mV and symmetrical 100 mM KCl concentrations in the outer and the inner compartment, lower trace: chrysospermin C channels at zero voltage and gradient of KCl (300 mM in the outer and 100 mM in the inner compartment). Calibrations bars: vertical: 10 pA, horizontal 2.5 s. Upward current corresponds to movement of positive ions from the inner compartment. Voltage is measured relative to the potential of the inner electrolyte compartment.

Thus, we can calculate from Fig. 2 that m, as the mean number of molecules within a conducting aggregate, amounts to ≈ 4, and from Fig. 3 that the value of α amounts to 0.85, i.e., less then moderate voltage dependence. This suggests that chrysospermin has a large partition coefficient in favour of the membrane phase, even in the absence of an electric field, probably due to its high hydrophobicity (in comparison with alamethicin molecule [9]). This feature can be interpreted in terms of the formation of non-gated ion channels.

The four homologous chrysospermins A (Ia), B (Ib), C (Ic), and D (Id) form cation selective ion channels in a comparable way. The channels are permeable to monovalent cations in the order Rb > Cs > K > Na > Li as determined by conductances of single channels. For chrysospermin B, for example, the channel conductances in 100 mM RbCl, CsCl, KCl, NaCl and LiCl (pH 6.2) amounted to 800, 710, 640, 525 and 240 pS. As shown in Figs. 4a and b, 6 and in Table 1, as well, the ratio of cation to anion transfer numbers (P(c)/P(a)) amounted to approx. 30 for each of the peptides. This suggests that they form ion channels of comparable structure.

Otherwise, there were considerable differences in overall conductance between Ia and Ic on the one side, and Ib and Id, on the other (Table 1).

The conductance of the ion channels formed by chrysospermin A (Ia) coincides well with the conductance

Table 1
Conductances of single channels in 100 mM KCl, and zero-current membrane voltages for 10-fold KCl gradient, as calculated with data obtained from plotting of $I-V$ curves of single channels at conditions of 3:1 gradient of KCl (Fig. 6)

Substance		Single-channel conductance in 100 mM KCl	Membrane voltage for 10:1 gradient of KCl concentration
Chrysospermin A	(Ia)	260 ± 50 pS	50 ± 5 mV
Chrysospermin B	(Ib)	640 ± 70 pS	57 ± 5 mV
Chrysospermin C	(Ic)	250 ± 50 pS	48 ± 5 mV
Chrysospermin D	(Id)	630 ± 70 pS	50 ± 5 mV

of chrysospermin C (Ic) channels (250 pS ± 50 pS in 100 mM KCl). The conductance of chrysospermin B (Ib) channels was similar to that of chrysospermin D (Id) channels (640 pS ± 70 pS in 100 mM KCl). This is also demonstrated in Fig. 5 by the amplitude histograms of chrysospermin C (Ic) and D (Id) showing that the most probable value of the former (Ic) is less than one half of the latter amounting to 18 pA. Estimation of mean open and closed time of the channels (with accuracy about ±15%) for each of the four peptides led to the following data: mean open times of A, B, C, and D channels were 1.0, 2.7, 1.2, and 2.5 s, respectively. The mean closed times were 2.5, 11.0, 4.5, and 9.3 s, respectively.

Single channel current traces for each of the four peptides were recorded at very low, even zero, membrane voltages (see, e.g., Figs. 4a,b and 6). In the case of alamethicin (II) the corresponding voltages are in the range of 100–150 mV [1]. This result discloses major differences in channel formation by chrysospermins and alamethicin.

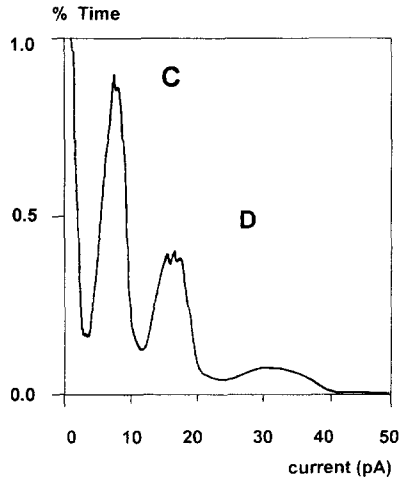

Fig. 5. Amplitude histogram associated with chrysospermins C and D peaks (see Fig. 4a and b), demonstrating that most probable amplitude of chrysospermin C single-channel current (−7 pA) is lower then that of chrysospermine D channel (−18 pA). Membrane voltage was −24 mV, 100 mM KCl.

4. Discussion

Each of the four homologous chrysospermins A, B, C, and D forms cation selective ion channels in lipid bilayer membranes, (see c.f. Figs. 4a and b: ratio of $P(o)/P(a) = 30$ for each of the peptides).

The tested chrysospermin peptides can be subdivided into two groups, A/C and B/D, respectively, in accordance to the conductance of the single channels.

Obviously, amino acid structures at position 15 (α-aminoisobutyric acid or isovaline) contribute in a particular manner to channel conductance. A comparably higher conductivity has been observed if molecule position 15 is occupied by isovaline.

Irrespective of the differences in conductivity of chrysospermins we may conclude that the observed high value of cation over anion selectivity (approx. 30), as a characteristic of all of the four chrysospermins, could be explained by the similarity of the polar interiors of the formed channels. This feature could be ascribed to comparable conformations of the helical peptide backbones and similar distances of the polar amide groups (1,2,3).

In contrast to alamethicin (II), current traces of the single channels were recorded for each of the four peptides even in the absence of membrane voltages but in presence of a membrane gradient of ions. This observation could be interpreted in terms of the formation of non-gated channels, since in the case of alamethicin (II) the corresponding voltages needed to channel formation range from 100 to 150 mV.

Support to this contention has been provided recently by the published results about trichorzianin TA IIIc (III) [14]) suggesting that the C-terminal tryptophanol contributes an additional dipole moment to the molecule which facilitates helical folding and channel formation even at low voltages.

The results shown in Fig. 6 suggest that channel conductivity of chrysospermins is determined mainly by the steric structure of the amino acids at position 15 (α-aminoisobutyric acid or isovaline). Obviously, the same changes at position 5 do not essentially influence channel conductance. This coincides well with recently published, results of Duclohier et al. [14] on trichorzianins. Moreover, differences in conductivity of chrysospermin channels are not connected with alterations of ion selectivity.

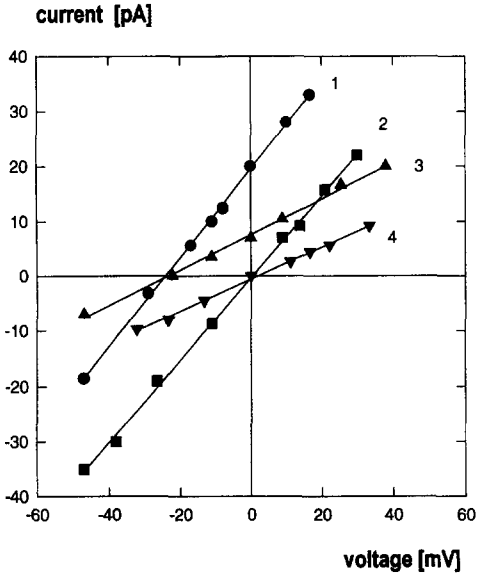

Fig. 6. Single-channel current–voltage ($I-V$) curves compared between chrysospermins C (▲; ▼, lines 3 and 4) and chrysospermins D (●; ■, lines 1 and 2) as recorded at voltage-clamp conditions. Current–voltage curves marked with ■ and ▼ symbols refer to the symmetrical 100 mM KCl concentrations in both compartments; ● and ▲ symbols – for 300/100 mM KCl gradient. Note: conductivities of C and D single channels are different, 270 pS and 640 pS, respectively, but cation to anion selectivity is the same. (The lines are fits by eye.)

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