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ION CHANNEL-FORMING PROPERTY OF TRICHOROVIN-XII, AN 11-RESIDUE PEPTAIBOL FROM THE FUNGUS *Trichoderma viride*, IN PLANAR LIPID BILAYER MEMBRANES

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Abstract: An 11-residue peptaibol, trichorovin-XII, isolated from the fungus *Trichoderma viride*, was found to form voltage-dependent and cation-selective ion channels in planar lipid bilayer membranes. The channels formed were classified into two types; a short-lived channel whose conductance level was not clearly distinguished and a long-lived channel whose conductance levels were 0.51 and 1.24 nS. Trichorovin-XII is the shortest channel-forming peptide of the peptaibol family so far reported.

Trichorovin-XII (TV-XII) [1] is a peptaibol [2] isolated from conidia of the fungus *Trichoderma viride*, together with trichocellins [3] and trichodecenins,[4] in the course of a search for antibacterial substances. TV-XII is a mixture of two similar components, TV-XIIa (main) and TV-XIIb (minor).

TV-XIIa: Ac-Aib-Asn-Ile-Ile-Aib-Pro-Leu-Leu-Aib-Pro-Iol
(Aib: α-aminoisobutyric acid, Iol: isoleucinol)
TV-XIIb: Ac-Aib-Asn-Lxx-Lxx-Aib-Pro-Lxx-Lxx-Aib-Pro-Lol
(Lxx: Ile or Leu, Lol: leucinol)

Peptaibols act as membrane modifiers, and have ion channel-forming ability in planar lipid bilayer membranes.[5] The electrical properties of the channels formed by 20-residue peptaibols can be interpreted in terms of a barrel-stave model,[6] in which a bundle of parallel α-helical rods forms a hydrophilic cylindrical pore. To span the *ca.* 3-nm-thick hydrophobic region of the lipid membrane, the α-helices should be at least 20 residues long. Unexpectedly, TV-XII, an 11-residue peptaibol, was found to form ion channels in planar lipid bilayer membranes. This paper describes the characteristics of the ion channels formed by TV-XII.

In a macroscopic examination, current-voltage curves were taken by imposing a triangular wave voltage (100 s per cycle) on a planar lipid bilayer membrane in 0.1 M $CaCl_2$ solution containing 1.0 to 2.5 μM TV-XII. The membrane was formed by painting a lipid solution [egg phosphatidylcholine (167.7 mg, Merck) and cholesterol (46.2 mg) in *n*-decane (20 ml)] on a *ca.* 1 mm diameter hole in a Teflon septum. When TV-XII was

added to one side of the membrane, symmetrical current-voltage (I-V) curves were obtained (Fig. 1a), while alamethicin, trichosporin-Bs and trichocellins usually provide asymmetrical I-V curves (Fig. 1b).[7] The I-V characteristics indicate that TV-XII forms voltage-dependent ion channels like alamethicin, trichosporin Bs and trichocellins, and that the channel formation is independent of the polarity of the applied voltage.

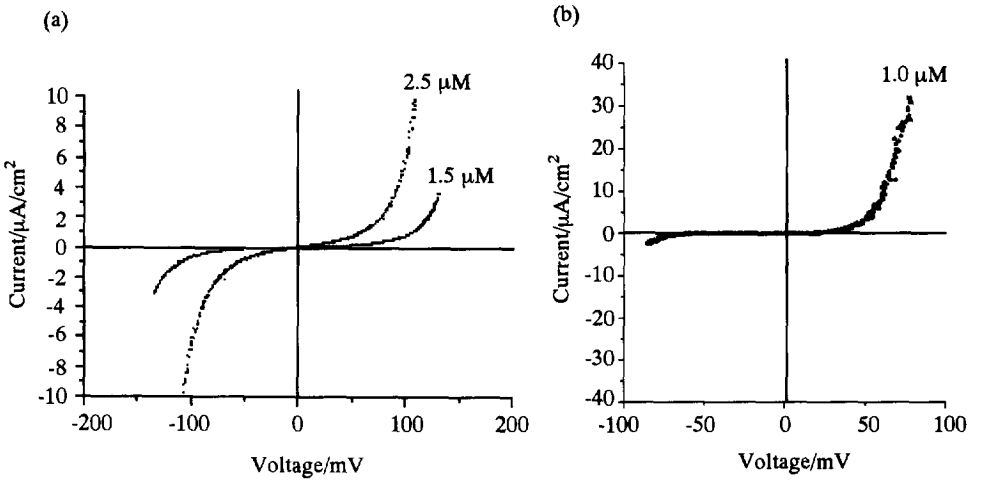

Fig. 1. Current-Voltage Curves Induced by Unilateral Addition of 1.5 μM and 2.5 μM TV-XII (a) and of 1.0 μM Trichocellin-A-II (b). Sequence of trichocellin-A-II: Ac-Aib-Ala-Aib-Ala-Aib-Ala-Gln-Aib-Leu-Aib-Gly-Aib-Aib-Pro-Val-Aib-Iva-Gln-Gln-Pheol (Iva: isovaline, Pheol: phenylalaninol)

In order to investigate further the properties of TV-XII ion channels, a microscopic (single ion channel) study was performed. Planar lipid bilayer membranes were formed by the folding method [8] with diphytanoylphosphatidylcholine (Avanti Polar Lipids). The electrolytes examined were 1 M KCl, 0.5 M $CaCl_2$, 0.2 M tetramethylammonium chloride (TMA-Cl) and 0.2 M tetraethylammonium chloride (TEA-Cl), whose cations all differ in radius. TV-XII was added to only one side (*cis* side) of the bilayer lipid membrane. The opposite side (*trans* side) was grounded.

With a 1 M KCl solution, a positive voltage applied to the *cis* side of the membrane gave only short-lived current spikes whose conductances could not be clearly resolved (Fig. 2a). On the other hand, a negative voltage applied to the *cis* side of the membrane caused long-lived discrete channel openings with distinct conductance levels 1 to 8, in addition to short-lived current spikes (Fig. 2b, c). The conductances of levels 1 and 2 were 0.51 nS and 1.24 nS, respectively, and those of levels 3 to 8 were equal to integral increments of that of level 2 (1.24 nS). This regularity of the conductance levels suggests that the channels corresponding to levels 3 ~ 8 result from simultaneous openings of the level 2 channel, differently from alamethicin and trichosporin B channels, which open in an uptake and release fashion.[6, 9] The results suggest that TV-XII forms two types of channels, which may involve different modes of aggregation.

In the case of 0.5 M $CaCl_2$ or 0.2 M TMA-Cl solutions, current steps were not observed, except for short-lived current spikes, even though a voltage up to ± 200 mV was applied. Furthermore, the TV-XII channel was inactive in 0.2 M TEA-Cl solution at both negative and positive polarities. Such single-channel

behavior with cations of different size suggests that both of the channels are cation-selective and that Ca^{2+} and TMA^{+} cannot permeate through the long-lived channels but can pass through the short-lived channels.

The CD measurements of TV-XII indicate that TV-XII takes a helical structure. Thus, as proposed for channel formation by other short helical peptides, mastparan and bombolitin,[5a] TV-XII might form channels by formation of long helical rods via head-to-tail dimerization of TV-XII helices (Fig. 3a) or by distortion of the bilayer to accommodate the length of TV-XII (Fig. 3b). TV-XII is the shortest channel-forming peptide of the peptaibol family so far reported.

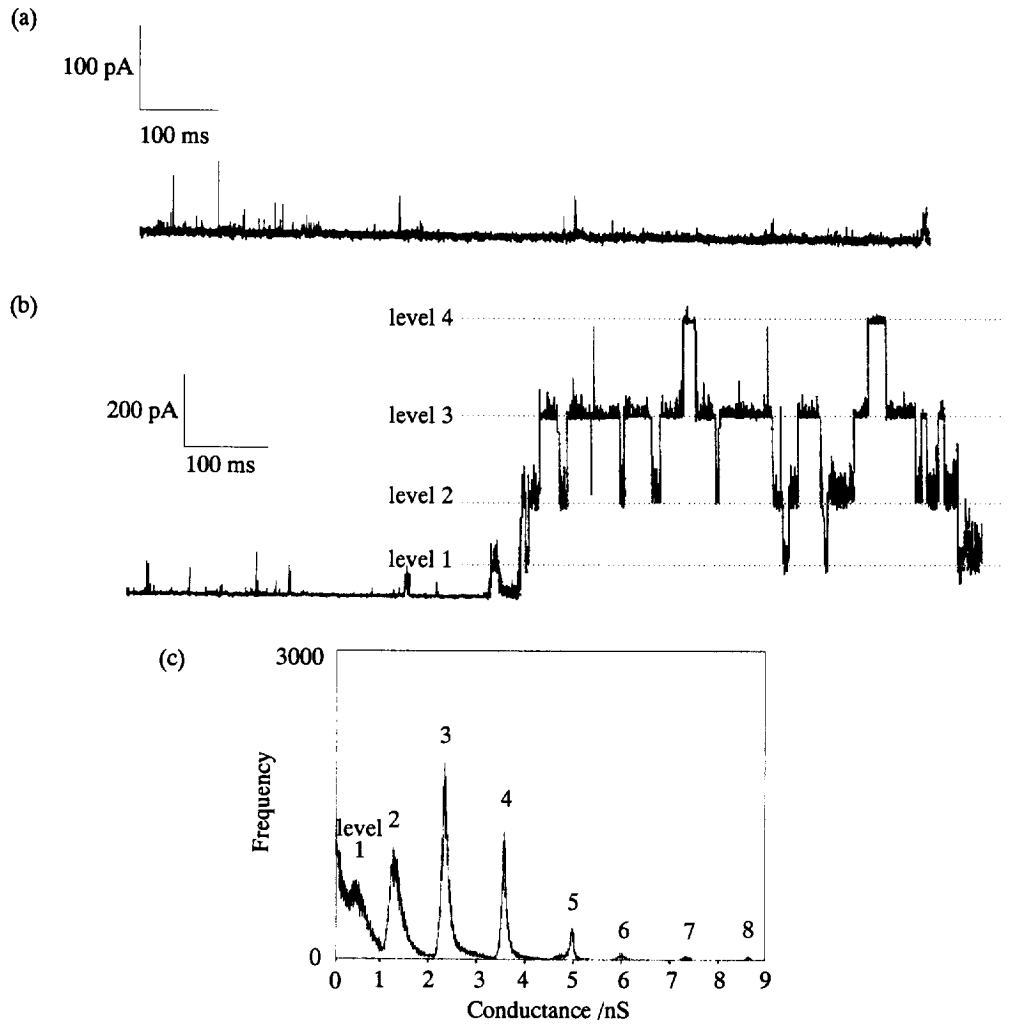

Fig. 2. Single-Channel Recordings (a, b) Measured in 1 M KCl Solution and Conductance Histogram (c) Derived from the Recording (b). TV-XII was present at 0.71 μM in the *cis* side of the membrane. The membrane potentials of the *cis* side of the membrane were +200 mV (a) and −185 mV (b).

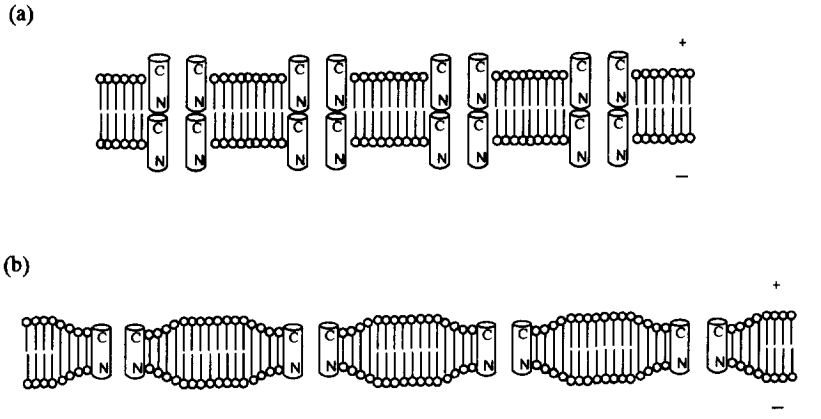

Fig. 3. Postulated Models, Head-to-Tail Dimerization (a) and Local Bilayer Distortion (b), for Channel Formation by TV-XII. Simultaneous openings of the channel were depicted in (a) and (b).

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