Ion-channel-forming and Catecholamine-releasing Activities of Elongated and Truncated Analogues of Trichosporin-B

Yasuo Nagaoka,[a] Akira Iida,[a] Takeshi Kambara,[a] Koji Asami,[b] Eiichi Tachikawa[c] and Tetsuro Fujita*[a]†

[a] *Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan*
[b] *Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan*
[c] *School of Medicine, Iwate Medical University, Morioka 020, Japan*

Examination of newly synthesized elongated and truncated analogues of the ion-channel-forming peptide, trichosporin-B-VIa (20 residues), for ion-channel-forming activity in lipid bilayer membranes and catecholamine secretion-inducing activity from adrenal chromaffin cells revealed that the natural compound has the optimal molecular length for both activities.

Trichosporin (TS)-B-VIa[1] is a peptaibol[2] isolated from the culture broth of *Trichoderma polysporum* (Link *ex* Pers.) Rifai (strain TMI 60146). TS-B-VIa forms voltage-gated ion channels in lipid bilayers,[3] as other peptaibols do.[4] In addition, TS-B-VIa induces Ca^{2+} influx into bovine adrenal chromaffin cells and causes Ca^{2+}-dependent catecholamine secretion from the cells.[5] Peptaibol ion channels are considered to consist of a bundle of transmembrane helical peptides.[6] Thus, the length of the helical peptides should be comparable to the thickness of the lipid bilayer membranes. In this paper, we report the effect of peptide length on these activities using seven TS-B-VIa analogues prepared by addition of a helicogenic amino acid, α-aminoisobutyric acid (Aib), or removal of residues at the N-terminus of TS-B-VIa (Table 1).

The derivatives were synthesized by the solution-phase method as described before[7] and purified by reversed-phase HPLC. The production of the desired peptides was confirmed by high-resolution (HR) FAB-MS (Table 1). As shown in Fig. 1, the CD spectra of the analogues in methanol were characteristic of right-handed helices. All their helical contents were lower than that of TS-B-VIa, which has a fully helical structure[8] (Table 1). The helicity of [−3] and [−4] was especially low, suggesting that the helices contained in these peptides are too short to form ion channels in the membranes.

TS-B-VIa and analogues were tested for channel-forming ability in diphytanoylphosphatidylcholine (diphyPC) membranes. The membranes were formed on a hole in a Teflon film sandwiched between two half cells according to the method of Montal and Muellar.[9] Peptides were added to one side (*cis* side) of the membranes. Current fluctuations due to channel opening and closure were observed only when the *cis* side was positive. TS-B-VIa exhibited multilevel channel behaviour with transitions between non-integral conductance sub-states [Fig. 2(*a*)], which might be explained by extension or contraction of the pore lumen caused by uptake or release of peptide monomers in a bundle of helices.[10]

The addition of one Aib residue, [+1], to TS-B-VIa did not alter the channel behaviour [Fig. 2(*b*)], whereas the addition of two and three Aib residues, [+2] and [+3], greatly reduced the channel lifetime [Figs 2(*c*) and (*d*)]. Conductance at each level decreased as the number of residues was increased.

The truncated analogues did not induce well-resolved channel currents, but voltage-dependent current bursts were still observed (Fig. 3). Contrary to our prediction, even [−3] and [−4], which are not long enough to span the membrane, induced characteristic current bursts. These current bursts should be caused by some mechanism other than that of the helix bundle model.

In diphyPC membranes, only TS-B-VIa and [+1] shows well-resolved channel behaviour, indicating a strict requirement of 20 or 21 residues for the formation of stable channels.

Incubation of chromaffin cells with the elongated derivatives ([+1], [+2], and [+3]; 5 μmol dm^{-3}) caused comparable catecholamine secretion (23, 27 and 23% of total cellular

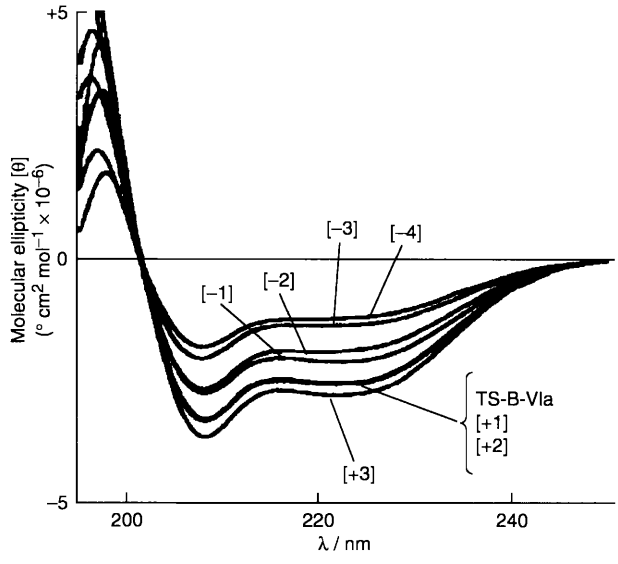

Fig. 1 CD spectra of TS-B-VIa and its derivatives in methanol (25 °C)

Table 1 Primary structures and characteristics of TS-B-VIa analogues[a]

Peptide	Position +3 +2 +1 1 2 3 4 5 —20[b]	HR FAB-MS[c] Calc.	$[M + H]^+$ Found, *m/z*	Helical content[d] (%)
[−4]	Ac-Aib—Pheol	1652.974	1652.976	65
[−3]	Ac-Ala-Aib—Pheol	1724.011	1724.014	70
[−2]	Ac-Aib-Ala-Aib—Pheol	1809.064	1809.055	90
[−1]	Ac-Ala-Aib-Ala-Aib—Pheol	1880.101	1880.095	90
TS-B-VIa	Ac-Aib-Ala-Aib-Ala-Aib—Pheol			100
[+1]	Ac-Aib-Aib-Ala-Aib-Ala-Aib—Pheol	2050.206	2050.201	93
[+2]	Ac-Aib-Aib-Aib-Ala-Aib-Ala-Aib—Pheol	2135.259	2135.266	89
[+3]	Ac-Aib-Aib-Aib-Aib-Ala-Aib-Ala-Aib—Pheol	2220.312	2220.317	93

[a] Aib = α-aminoisobutyric acid, Pheol = phenylalaninol. [b] The sequence between positions 6 and 19 is (-Aib-Gln-Aib-Ile-Aib-Gly-Leu-Aib- Pro-Val-Aib-Aib-Gln-Gln-). [c] FAB-MS was performed on a JEOL HX-110. [d] The helical contents were estimated from the ellipticity per residue at 207 and 221 nm. The relative values with respect to that of TB-B-VIa (100%) are shown.

catecholamine, respectively) to that of TS-B-VIa (34%). In contrast, catecholamine-releasing activity was reduced considerably by truncation. The [−**1**], [−**2**], [−**3**], and [−**4**] peptides were inactive at 5 μmol dm^{-3}, whereas at 20 μmol dm^{-3} they induced the secretion of 33, 11, 1, and 1% of total cellular catecholamine, respectively.

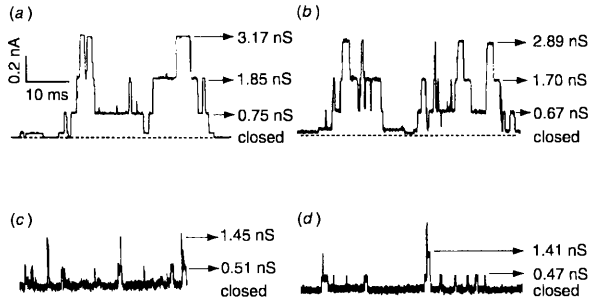

Fig. 2 Single-channel recordings of TS-B-VIa and its elongated derivatives: (*a*) 4 nmol dm^{-3} TS-B-VIa at 270 mV; (*b*) 8 nmol dm^{-3} [+**1**] at 265 mV; (*c*) 5 nmol dm^{-3} [+**2**] at 255 mV; (*d*) 4 nmol dm^{-3} [+**3**] at 210 mV

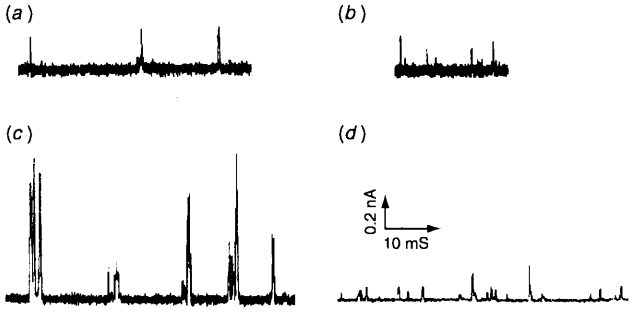

Fig. 3 Single-channel recordings of truncated derivatives: (*a*) 20 nmol dm^{-3} [−**1**] at 265 mV; (*b*) 30 nmol dm^{-3} [−**2**] at 265 mV; (*c*) 50 nmol dm^{-3} [−**3**] at 265 mV; (*d*) 70 nmol dm^{-3} [−**4**] at 265 mV

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† *New address*: Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata, Osaka 573-01, Japan.

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