EFFECT OF LIPOPHILICITY OF TRICHOSPORIN-Bs ON ION-CHANNEL FORMATION AND CATECHOLAMINE-RELEASING ACTIVITY

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A series of peptaibols, trichosporin (TS) -Bs, induced voltage-activated conductance in lipid bilayer membranes and catecholamine secretion from bovine adrenal chromaffin cells. The order of activities is the same as that of the lipophilicity rank of TS-Bs derived from the reversed-phase HPLC capacity factors.

KEY WORDS trichosporin; *Trichoderma polysporum*; peptaibol α -aminoisobutyric acid; ion-channel; catecholamine

Trichosporin(TS)-Bs¹⁾ are peptaibols²⁾ isolated from the culture broth of *Trichoderma polysporum* (Link ex Pers.) Rifai (strain TMI 60146). TS-Bs form voltage-gated ion channels in lipid bilayers,³⁾ as other peptaibols do.⁴⁾ In addition, TS-Bs induce Ca^{2+} influx into bovine adrenal chromaffin cells and cause Ca^{2+} -dependent catecholamine secretion from the cells.⁵⁾ Here we describe the effect of peptide lipophilicity on the above activities in the case of five TS-B components (TS-B-Ia, -IIIa, -V, -VIa, and -VIb) shown in Table 1.

TABLE 1. Primary Structures of Trichosporin-Bs^{a)}

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
TS-B-Ia	Ac	Aib	Ala	Ser	Ala	Aib	Aib	Gln	Aib	Leu	Aib	Gly	Leu	Aib	Pro	Val	Aib	Aib	Gln	Gln	Pheol
TS-B-IIIa	Ac	Aib	Ala	Ala	Ala	Aib	Aib	Gln	Aib	Leu	Aib	Gly	Leu	Aib	Pro	Val	Aib	Aib	Gln	Gln	Pheol
TS-B-V	Ac	Aib	Ala	Ala	Ala	Aib	Aib	Gln	Aib	Ile	Aib	Gly	Leu	Aib	Pro	Val	Aib	Aib	Gln	Gln	Pheol
TS-B-VIa	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Ile	Aib	Gly	Leu	Aib	Pro	Val	Aib	Aib	Gln	Gln	Pheol
TS-B-VIb	Ac	Aib	Ala	Ala	Ala	Aib	Aib	Gln	Aib	Ile	Aib	Gly	Leu	Aib	Pro	Val	Aib	Iva	Gln	Gln	Pheol

^{a)} Aib = α -aminoisobutyric acid, Pheol = phenylalaninol, Iva = isovaline.

Fig. 1 shows the current-voltage (I-V) relationships of lipid bilayer membranes, of which one side (cis side) was exposed to a solution containing TS-B. When a trans-membrane potential was applied, a TS-B-activated current occurred only under the condition that the cis side was positive. These currents were considered to be due to the openings of a large number of voltage-activated channels.⁴⁾ The exponential branches of the currents for each TS-B at the same concentrations (50 nM-125 nM) were translated to high field in the order of TS-B-VIb, -VIa, -V, -IIIa, and -Ia. This

order is identical to that of capacity factor values (k' , a measure of lipophilicity⁷⁾) for TS-Bs, determined from their retention times on reversed-phase HPLC. This shows that a more lipophilic TS-B can form channels at a lower membrane voltage at the same concentration of TS-Bs. Furthermore, linear relationships were observed between $\log k'$ and the logarithmic value of the

Fig.1. Current -Voltage (I-V) Curves Induced by 75 nM TS-Bs in Planar Lipid Bilayers

The lipid bilayer membranes were formed from diphytanoylphosphatidylcholine according to the method of Montal and Mueller.⁶⁾ The electrolyte solution was 1M KCl, unbuffered. A triangular voltage wave (100 s / period) was applied to the membranes.

Fig. 2. Double-Logarithmic Plots of k' Versus the Conductance (G) of the Membranes at 120 mV with TS-Bs at the Concentration of 50, 75, or 100 nM

The k' values were determined from the retention time of TS-Bs (t) on HPLC using a phenyl column with the mobile phase MeOH-H₂O (85:15) as $k' = (t - t_0)/t_0$, where t_0 is the time for elution of unretained NO₃³⁻ ion.

■, TS-B-VIb, ●, -VIa, △, -V, ○, -IIIa, □, -Ia.

Fig.3. Effect of 5 μ M TS-B-Ia, -IIIa, -V, -VIa, and -VIb on Catecholamine Secretion from Adrenal Chromaffin Cells

The cells were incubated for 5 min at 37°C with TS-Bs. Catecholamine secretion is shown as a percentage of total cellular catechol-amine content. Data are means $\pm$ standard deviations from four experiments. *, $p < 0.001$, significantly different from the control (Cont).

Fig.4. Relationship between $\log$ [Catecholamine-Releasing Activity (BA)] of TS-Bs and Their Relative Hydrophobicity ($\log k'$)

Catecholamine-releasing activity was obtained as shown in Fig.1. The k' value was determined as shown in Fig.2.

membrane conductance at 120 mV induced by 50, 75, and 100 nM TS-Bs with high correlation coefficients ($r = 0.994, 0.992, \text{ and } 0.987$, respectively) (Fig. 2). These results indicate that the extent of voltage activation of the TS-B-induced-currents and membrane conductance depends on the lipophilicity of TS-Bs.

Incubation of chromaffin cells with TS-Bs (5 μM) caused an increase in the secretion of catecholamine in the order of TS-B-VIb > -VIa > -V > -IIIa > -Ia (Fig. 3). A double-logarithmic plot of k' against catecholamine-releasing activity (BA) induced by TS-Bs is linear with a high correlation coefficient ($r = 0.941$) (Fig. 4). This result indicates that the catecholamine-releasing activity also depends on the lipophilicity of TS-Bs.

The five TS-Bs differ from each other in replacements at positions 3 (Ser, Ala or Aib), 9 (Leu or Ile), and 17 (Aib or Iva). These changes alter the lipophilicity of the TS-B-molecules and cause differences in their activities in the membranes. We conclude that lipophilicity plays a key role in determining the activities of TS-Bs. Furthermore, the results obtained herein suggested that both activities, the influx of external Ca^{2+} into the adrenal chromaffin cells and the conductance increment of lipid bilayers by the peptaibols, are caused by the same mechanism, i.e., the formation of voltage-activated ion-channels in the biomembranes and artificial membranes.

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