Role of Gln^7 in the ion-channel-forming properties of the peptaibol trichosporin-B-VIa

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Replacement of the Gln^7 residue, which is well conserved among ion-channel-forming peptaibols, by an Ala in trichosporin-B-VIa (TS-B-VIa = AcUAUAUUQUIUGL-UPVUUQQPheol; U = α-aminoisobutyric acid, Pheol = phenylalaninol) alters neither the multilevel nature of the TS-B-VIa channel nor its conductance, but abolished the rectification of the membrane current characteristic of the TS-B-VIa channel.

In the ion-channel-forming peptaibols, which are linear amphiphilic peptides rich in α-aminoisobutyric acids (Aib) and having a *C*-terminal aminoalcohol moiety, the Gln residue at position 7 is well conserved.[1] The polar side chain of Gln^7 is proposed to be directed towards the centre of the ion-channel pore, which consists of a bundle of transmembrane helical peptaibols. It is considered to be important for the formation of a hydrophilic environment to the pore and for the stabilization of the bundle by means of polar interactions with water molecules in the channel pore.[2]

Trichosporin-B-VIa (TS-B-VIa)[3] is a peptaibol 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,[4] as do other peptaibols.[1] In order to investigate the influence of Gln^7 in TS-B-VIa on the ion-channel-forming activity, the Gln^7 residue was replaced by a nonpolar helicogenic amino acid, Ala, to make [Ala^7]TS-B-VIa. The sequence of [Ala^7]TS-B-VIa is as follows: Ac-Aib-Ala-Aib-Ala-Aib-Aib-Ala-Aib-Ile-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol, where Ac is acetyl and Pheol is phenylalaninol.

[Ala^7]TS-B-VIa was synthesized by the condensation of an *N*-terminal fragment (positions 1–15) to a *C*-terminal fragment (positions 16–20). The *N*-terminal fragment was prepared by solid-phase synthesis using Fmoc-amino acid fluorides as acylating agents.[5] Synthesis of the *C*-terminal fragment and the fragment condensation were carried out by the solution-phase method as described previously.[6] [Ala^7]TS-B-VIa was purified from the crude products, including the [D-Val^{15}] isomer, by reversed-phase HPLC. The production of [Ala^7]TS-B-VIa was confirmed by high-resolution FAB-MS (calc. 1908.137 [M + H]$^+$, found 1908.132) and chiral-phase amino acid analysis.

CD spectra and amide coupling constants ($^3J_{NH\text{-}\alpha H}$) for [Ala^7]TS-B-VIa measured in methanol were characteristic of helical structure and almost identical to those of TS-B-VIa, suggesting that the Gln^7 → Ala^7 substitution does not alter the backbone structure of TS-B-VIa.[7,†]

Microscopic current fluctuations and macroscopic current–voltage curves were measured with diphytanoylphosphatidylcholine membranes, which were prepared on a *ca.* 100 μm hole in a Teflon film sandwiched between two half cells as described by Montal and co-worker.[8] Peptides were always added to one side (*cis* side) of the membranes. [Ala^7]TS-B-VIa induced multi-level current fluctuations, as did TS-B-VIa [Fig. 1(*a*)]. Table 1 shows the channel parameters for [Ala^7]TS-B-VIa and TS-B-VIa at 220 mV. The conductance and opening probability calculated for each substate of the [Ala^7]TS-B-VIa channel are comparable to those of the TS-B-VIa channel. Channel lifetimes of higher substates were reduced by the Gln^7 → Ala^7 substitution, but the reductions are not large. Channel lifetimes were reduced more drastically by the truncation, elongation or

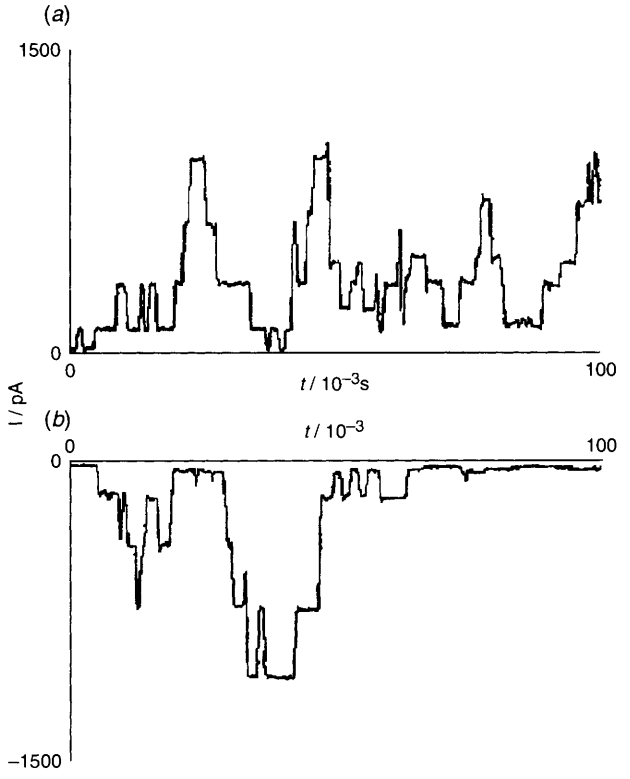

Fig. 1 Single-channel recordings of 5 × 10^{-9} mol dm^{-3} [Ala^7] TS-B-VIa doped membranes clamped at (*a*) +220 mV or (*b*) −235 mV in unbuffered 1 mol dm^{-3} KCl solution

Table 1 Single-channel parameters of [Ala^7] TS-B-VIa (4 × 10^{-9} mol dm^{-3}) and TS-B-VIa (5 × 10^{-9} mol dm^{-3}) at +220 mV

	Conductance/nS		Open probability[a]		Mean life-time[b]/ 10^{-3} s	
Level	[Ala^7]	TS-B-VIa	[Ala^7]	TS-B-VIa	[Ala^7]	TS-B-VIa
1	0.07	0.06	0.35	0.41	0.45	0.26
2	0.53	0.57	0.37	0.33	0.79	0.75
3	1.55	1.64	0.19	0.18	0.36	0.72
4	2.90	3.00	0.08	0.07	0.30	0.73
5	4.41	4.52	0.01	0.01	n.d.[c]	n.d.[c]

[a] Open probability of each level within bursts, closed state excluded. [b] Mean life-time (τ) was obtained by fitting the equation, $P_t = e^{-t/\tau}$, to the plot of lifetime probability (P_t) distribution for each substate. [c] N.d. = not determined.

Pro[14] → Aib[14] substitution of TS-B-VIa, as examined in our previous studies.[4,9] Unexpectedly, the results suggest that the Gln[7] side chain does not play an important role in determining the channel conductance and stability.

The most significant difference caused by the substitution was in the voltage dependence of the channel formation. The current fluctuations induced by TS-B-VIa were observed only when the *cis* side was positive, while those induced by [Ala[7]]TS-B-VIa were observed at both positive and negative voltages (Fig. 1). The current induced by [Ala[7]]TS-B-VIa at a negative voltage has similar channel parameters to that induced at a positive voltage, indicating that these channels have an identical structure. This suggests that the channel at negative voltage was formed by [Ala[7]]TS-B-VIa molecules which had translocated to the *trans* side of the membrane.

Macroscopic current–voltage (I–V) curves are compared in Fig. 2. TS-B-VIa induced asymmetric I–V curves in which exponential currents were observed above critical positive voltages. In the [Ala[7]]TS-B-VIa-doped membrane, the I–V curves were symmetrized and current due to voltage-independent conductance developed. When the dipole reorientation model[10] was applied to this gating event, it was found that the charge on an [Ala[7]]TS-B-VIa molecule moves 0.28 times as far as that on a TS-B-VIa molecule.‡ The result suggests that the orientation of the molecules in the membranes during the gating event was different between these two peptides.

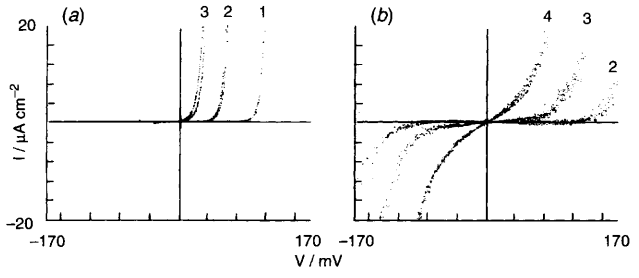

Fig. 2 Macroscopic current–voltage curves of (*a*) TS-B-VIa and (*b*) [Ala[7]] TS-B-VIa at various concentrations: 1.0×10^{-7} mol dm^{-3} (curve 1), 1.5×10^{-7} mol dm^{-3} (curve 2), 2.0×10^{-7} mol dm^{-3} (curve 3) and 3.0×10^{-7} mol dm^{-3} (curve 4). Triangle-wave voltages (0.01 Hz) were applied to the membranes in unbuffered 1 mol dm^{-3} KCl solution.

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Footnotes

† Molecular ellipticities, θ (deg cm^2 dmol^{-1}), for [Ala[7]]TS-B-VIa and TS-B-VIa at 208 and 222 nm in methanol (25 °C): -3.348×10^5 and -2.591×10^5 ([Ala[7]]TS-B-VIa), -3.415×10^5 and -2.639×10^5 (TS-B-VIa). N*H* chemical shifts and the $^3J_{NH\text{-}\alpha H}$ values for [Ala[7]]TS-B-VIa were determined by the same procedure as previously described:[6] $\delta_H(CD_3OH$, 293 K, 600 MHz, 5×10^{-3} mol dm^{-3}) 8.468 (s, Aib[1]), 8.310 (d, *J* 4.3 Hz, Ala[2]), 7.648 (s, Aib[3]), 7.63 (d, *J* 5.7 Hz, Ala[4]), 7.986 (s, Aib[5]), 7.972 (s, Aib[6]), 7.773 (d, *J* 4.4 Hz, Ala[7]), 8.184 (s, Aib[8]), 7.575 (d, *J* 5.9 Hz, Ile[9]), 8.323 (s, Aib[10]), 8.386 (t, *J* 4.8, 6.4 Hz, Gly[11]), 8.085 (d, *J* 7.6 Hz, Leu[12]), 8.410 (s, Aib[13]), 7.64 (d, *J* 7.8 Hz, Val[15]), 7.602 (s, Aib[16]), 7.834 (s, Aib[17]), 7.804 (d, *J* 5.3 Hz, Gln[18]), 7.893 (d, *J* 7.4 Hz, Gln[19]), 7.335 (d, *J* 9.1 Hz, Pheol[20]).

‡ The effective gating charge, α, calculated from the equation described by Hall *et al.*[11] was 0.50 for TS-B-VIa and 0.14 for [Ala[7]]TS-B-VIa. If these peptides have the same dipole moment, the ratio of α in these peptides (1 : 0.28) represents the ratio of the distance moved by the charge.

References

1 M. S. P. Sansom, *Eur. Biophys. J.*, 1993, **22**, 105, and references cited therein.
2 R. O. Fox and F. M. Richards, *Nature*, 1982, **300**, 325.
3 A.Iida, M. Okuda, S. Uesato, Y. Takaishi, T. Shingu, M. Morita and T. Fujita, *J. Chem. Soc., Perkin Trans. 1*, 1990, 3249.
4 Y. Nagaoka, A. Iida, T. Kambara, L. Neoh, T. Fujita, K. Asami, K. Asaka, E. Tachikawa and T. Kashimoto, *Peptide Chemistry 1993*, ed. Y. Okada, Protein Research Foundation, Osaka, 1994, pp. 361–364.
5 H. Wenschuh, M. Beyermann, H. Haber, J. K. Seydel, E. Krause, M. Bienert, L. A. Carpino, A. El-Faham and F. Albericio, *J. Org. Chem.*, 1995, **60**, 405.
6 Y. Nagaoka, A. Iida and T. Fujita, *Chem. Pharm. Bull.*, 1994, **42**, 1258.
7 Y. Nagaoka, A. Iida, E. Tachikawa and T. Fujita, *Chem. Pharm. Bull.*, 1995, **43**, 1119.
8 M. Montal and P. Mueller, *Proc. Natl. Acad. Sci. USA*, 1972, **69**, 3561.
9 Y. Nagaoka, A. Iida, T. Kambara, K. Asami, E. Tachikawa and T. Fujita, *J. Chem. Soc., Chem. Commun.*, 1995, 2203.
10 G. Boheim, W. Hanke and G. Jung, *Biophys. Struct. Mech.*, 1983, **9**, 181.
11 J. E. Hall, I. Vodyanoy, T. M. Balasubramanian and G. R. Marshall, *Biophys. J.*, 1984, **45**, 233.

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