Tetrahedron Letters 55 (2014) 728–731

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Control of the cytotoxicity of dansylated polytheonamide mimic, an artificial peptide ion channel, by modification of the N-terminal structure

Hiroaki Itoh, Shoko Matsutaka, Takefumi Kuranaga, Masayuki Inoue *

Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

ARTICLE INFO

Article history:
Received 29 October 2013
Revised 28 November 2013
Accepted 3 December 2013
Available online 8 December 2013

Keywords:
Cytotoxic agents
Natural products
Structure–activity relationships
Peptides

ABSTRACT

We demonstrate that the cytotoxicity of dansylated polytheonamide mimic (**2**) is controlled by chemical modification of its N-terminal structure. Dansylated polytheonamide mimic (**2**) is an ion channel peptide which displays potent cytotoxicity against P388 mouse leukemia cells (IC_{50} = 12 nM). To modulate its cytotoxicity, three analogues of **2**, possessing distinct N-terminal structures with different hydrophobicities, were synthesized and their cytotoxicities were evaluated. This focused structure–activity relationship study unveiled that the cytotoxicity of **2** is enhanced 10-fold by simply changing its N-terminal 5,5-dimethyl-2-oxohexanamide to the more hydrophobic palmitamide. The data obtained here provide new understanding for the functional control of the artificial ion channel peptide **2**.

Polytheonamide B (**1**) is an ion channel forming peptide that displays extraordinarily potent cytotoxicity against P388 mouse leukemia cells (IC_{50} = 0.098 nM).[1] The 48-mer peptide **1** possesses a D,L-alternating amino acid sequence with numerous non-proteinogenic amino acids (Fig. 1)[2,3] This unusual peptide sequence folds into a $\beta^{6.3}$-helix in a hydrophobic environment;[4] the resulting tubular structure is believed to function as a transmembrane ion channel in a lipid bilayer.[5] Motivated by these unusual features, we launched a research program to investigate the structure and function of **1**, and recently accomplished the total synthesis[6] and a structure–activity relationship study of **1**.[7] Furthermore, artificial peptide **2**, designated as dansylated polytheonamide mimic, was designed as a simplified analogue of **1**. The synthesis and functional analysis of **2**[8] were recently achieved.[9,10] Mimic **2**, which differs from the parent natural product **1** by six amino acid residues, was demonstrated to form an ion conducting channel in a lipid bilayer and exhibited potent cytotoxicity against P388 mouse leukemia cells (IC_{50} = 12 nM), although its toxicity was approximately 100-times weaker than that of **1**.[8]

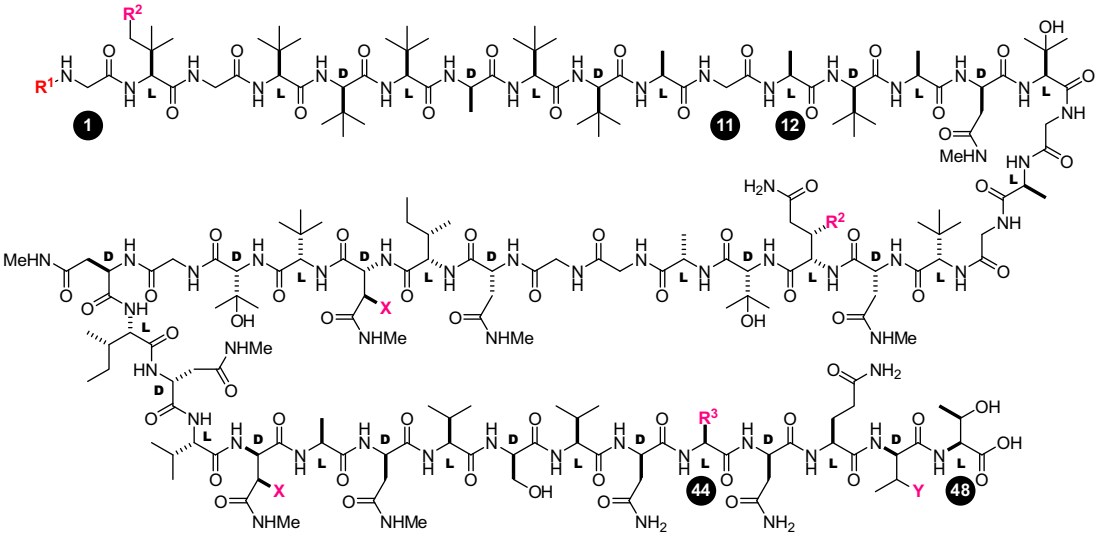
To create artificial peptides with more potent activities, we decided to enhance the cytotoxicity of **2** by structural modification. Here we report the synthesis and biological evaluation of N-terminal modified analogues of **2**, and discuss the strong correlation between the cytotoxicity and overall hydrophobicity. These results provide a new design principle for functional control of artificial cytotoxins based on large polypeptide structures.

The 48 amino acid sequence of dansylated polytheonamide mimic **2** is capped at the N-terminus with 5,5-dimethyl-2-oxohexanoate (Ncap). The hydrophobic Ncap of **2** was hypothesized to be the critical substructure for its cytotoxicity because the hydrophobic moiety would be required for effective insertion into the cell membrane, where **2** would exert its cytotoxicity by forming an ion channel. To undertake experimental verification of this hypothesis, we synthesized palmitamide **3** (Fig. 1), amine **4**, and trimethylammonium derivative **5**, which were intended to possess different hydrophobicities.

Three analogues **3**–**5** were chemically constructed through fully-protected 48-mer peptide **10** (Scheme 1), which was prepared by condensation between residue 1–11 (**7**) and residue 12–48 (**9**). Construction of 37-mer peptide **9** was attained by a single automated solid-phase peptide synthesis as previously reported.[8] Fmoc-protected 11-mer peptide **7** was synthesized from Fmoc-Gly-Wang resin **6**[11] using Fmoc-based chemistry[12] with HATU/HOAt activation.[13] Automated peptide chain elongation and cleavage from the resin produced desired peptide **7** (20% overall yield from **6**), which was converted to the corresponding thioester **8** using a reagent combination of $HS(CH_2)_2CO_2Et$, HOBt, and *N*,*N*′-diisopropylcarbodiimide in 82% yield. Then, the two fragments **8** and **9** were coupled under Ag-mediated coupling conditions.[14] When thioester **8** and amine **9** were treated with $AgNO_3$ and HOOBt in DMF/THF at 50 °C, the full-length 48-mer polyamide **10** was obtained. Finally, peptide **10** was treated with

* Corresponding author. Tel.: +81 3 5841 1354; fax: +81 3 5841 0568.
E-mail address: inoue@mol.f.u-tokyo.ac.jp (M. Inoue).

0040-4039/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2013.12.007



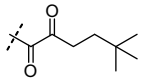
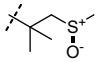
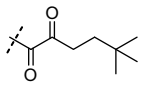
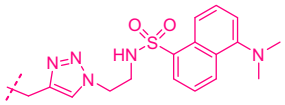
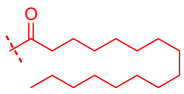
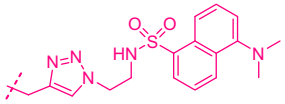
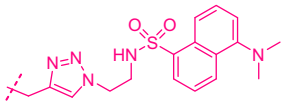
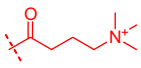
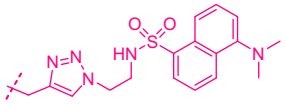
compounds	R^1	R^2	R^3	X	Y
polytheonamide B					
Ncap-[1-48]-OH (**1**)		Me		OH	(*R*)-OH
dansylated polytheonamide mimic					
Ncap-[1-48]-OH (**2**)		H		H	(*S*)-OH
newly synthesized compounds					
$CH_3(CH_2)_{14}CO$-[1-48]-OH (**3**)		H		H	(*S*)-OH
H-[1-48]-OH (**4**)	H	H		H	(*S*)-OH
$Me_3N^+(CH_2)_3CO$-[1-48]-OH (**5**)		H		H	(*S*)-OH

Figure 1. Structures of polytheonamide B (**1**), dansylated polytheonamide mimic (**2**), and newly synthesized analogues **3**–**5**. The sequences are abbreviated as R–[X–Y]–Z (R = functional group attached to the N-terminal amine, X = N-terminal residue number, Y = C-terminal residue number, Z = functional group attached to the C-terminal carboxylic acid). The residue numbers of **1** are written as plain text (R–[X–Y]–Z) and those of **2**–**5** are underlined (R–[X–Y]–Z).

piperidine/DMF to remove the N-terminal Fmoc group, then subsequently exposed to TFA/*i*-Pr_3SiH[15]/H_2O (95:2.5:2.5) to realize global deprotection (the eight Tmb, four Tr, and three *t*-Bu groups), successfully delivering amine **4** (20% overall yield from 37-mer peptide **9**). Palmitamide **3** and trimethylammonium derivative **5** were transformed from **4** by applying the corresponding mixed anhydrides in 57 and 23% yields, respectively.

Having efficiently synthesized **3**–**5**, the hydrophobicities of polytheonamide B (**1**), mimic **2**, and the three analogues **3**–**5** were compared by accessing their octanol/water partition coefficients (log*P*) using reversed phase HPLC.[16] First, the retention times of the standard samples (acetoanilide, thymol, biphenyl, and phenanthrene) with known log*P* values (1.0, 3.3, 4.0, and 4.5, respectively)[17] were measured using an ODS column and gradient elution of *i*-PrOH/water as mobile phase.[18] Next, polytheonamide B (**1**) and peptides **2**–**5** were eluted under the same HPLC conditions to deduce their log P values by comparison of their retention times with the standard samples.[19] As shown in Table 1, the log P value of the parent natural product **1** (4.5) was larger than that of polytheonamide mimic **2** (3.8), while replacement of 5,5-dimethyl-2-oxohexanamide of **2** with palmitamide in **3** increased the log*P* value from 3.8 to 4.9. On the other hand, amine **4** and trimethylammonium derivative **5** had lower hydrophobicity than **2** (log*P* = 3.2 and 2.6, respectively). Significant differences in the log*P* values of peptides **2**–**5**, whose structures are same except at the N-terminus, clarified that the N-terminal structure is the crucial factor in controlling the total polarity of this series of peptides.

Next, the cytotoxicities of **1** and mimics **2**–**5** against P388 mouse leukemia cells were evaluated using the XTT method (Table 1).[20,21] Although they were less toxic than the exceptionally bioactive natural product **1**, all the mimics exhibited potent cytotoxicities. Most importantly, palmitamide **3** had 10-fold stronger toxicity (IC_{50} = 1.0 nM) than the original mimic **2**, and thus was only 10-times less toxic than **1**. Not unexpectedly, amine **4** and trimethylammonium derivative **5** displayed 2-fold and 7-fold lower cytotoxicities than **2** (IC_{50} = 25 nM and 83 nM, respectively).[8]