Configuration of Amino Acid Residues for Stapling Bim Peptide Affects Both the Secondary Structure and Intracellular Bioactivity

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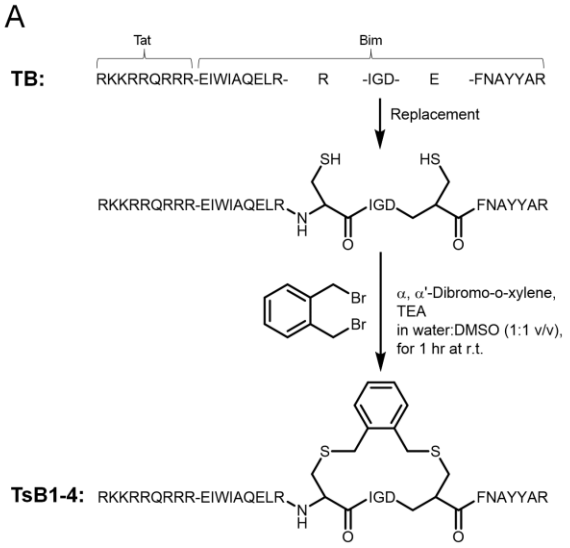
Stapling of a functional peptide is expected to stabilize its secondary structure and thereby improve its intracellular activity. However, regarding the cyclic portion of their stapling, there are no reports discussing the influence of the configuration of stapled amino acid residues on the secondary structure and the intracellular activity. To clarify the influence, we synthesized an apoptosis-inducing peptide, Bim (**B**), and four stapled Bim peptides that introduced Cys with different configurations at positions i and i+4 and cyclized the peptides with dibromoxylene, referring to the synthesis by Peraro *et al*.[1] (**sB1**, **sB2**, **sB3** and **sB4**; LL-, LD-, DL- and DD-configuration, respectively; Figure 1). When the secondary structure of these peptides was assessed by CD spectra, clear differences in secondary structure were observed depending on the configuration of Cys in the peptides. Their helix content was in the order of **sB1** > **B** > **sB4** ~ **sB3** (**sB2** showed a different structure from the α-helix). In addition, when the intracellular apoptotic activity was assessed using Tat-conjugated stapled Bim peptides (**TsB** series), their apoptosis efficiency was in the order of **TsB1** > **TB** > **TsB4** ~ **TsB3**. These results indicated that the configuration of Cys in the stapled Bim peptide affected the secondary structure and the intracellular activity of the peptide, and that there was a correlation between the two.



B

	Sequence
B	EIWIAQELR-R -IGD-E -FNAYYAR
sB1	EIWIAQELR-C*-IGD-C*-FNAYYAR
sB2	EIWIAQELR-C*-IGD-c*-FNAYYAR
sB3	EIWIAQELR-c*-IGD-C*-FNAYYAR
sB4	EIWIAQELR-c*-IGD-c*-FNAYYAR
TB	RKKRRQRRR-EIWIAQELR-R -IGD-E -FNAYYAR
TsB1	RKKRRQRRR-EIWIAQELR-C*-IGD-C*-FNAYYAR
TsB2	RKKRRQRRR-EIWIAQELR-C*-IGD-c*-FNAYYAR
TsB3	RKKRRQRRR-EIWIAQELR-c*-IGD-C*-FNAYYAR
TsB4	RKKRRQRRR-EIWIAQELR-c*-IGD-c*-FNAYYAR
Tmr-sB1	Tmr- EIWIAQELR-C*-IGD-C*-FNAYYAR
Tmr-TsB1	Tmr-RKKRRQRRR-EIWIAQELR-C*-IGD-C*-FNAYYAR

Figure 1. (A) Schematic illustration of synthesis of stapling Bim peptide by o-dibromoxyene through two Cys residues in the peptide. (B) Sequence of Bim peptide (**B**), stapled Bim (**sBn**; n = 1-4), Bim conjugated with a cell-penetrating peptide Tat (**TB**), stapled Bim conjugated with Tat (**TsBn**; n = 1-4), and fluorescent dye Tmr-labeled **sB1** (**Tmr-sB1**) and **TsB1** (**Tmr-sB1**). Capital letters showed L-configurated amino acid units and small letters showed D-configurated amino acid units. Asterisk shows the Cys residue stapled between the thiol groups and dibromoxylene. The N-terminal of all peptides is a free amino (H_2N-) group, and the C-terminal is a primary amide (-$CONH_2$) group.

References

[1] Peraro, L.; Zou, Z.; Makwana, K. M.; Cummings, A. E.; Ball, H. L.; Yu, H.; Lin, Y. Levine, B,; Kritzer, J. A. *J. Am. Chem. Soc.* **2017**, 139, 7792-7802.