Secondary Structure of Peptide Is Affected by Chain Length of an Alkyl Cross-linker Used for Stapling

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We are currently investigating improvement of stability of the secondary structure of a functional peptide (**Bim**) and a peptide hybrid for intracellular delivery of the functional peptide (**E/K**). The **Bim** peptide induces apoptosis by interacting with Bax proteins. The **E/K** hybrids can also deliver functional peptides into cells by using a cell-penetrating peptide. Shortening of these peptides is useful because they are easy to synthesize and have low toxicity, but it is difficult to form complexes because of the difficulty in taking up the α-helix structure. Therefore, we aim to use short amino acid sequences to form effective and small complexes to facilitate the interaction between **Bim** peptides and Bax proteins and the formation of **E/K** hybrids. We have studied the following three aspects to achieve this objective. These are: **a.** stabilization of the secondary structure by stapling, **b.** shortening of the amino acid sequence, and **c.** enhancement of interaction by introducing unnatural amino acids. For **Bim** peptides, **a** and **b** have been achieved in previous studies[1], and **a** + **b** approach is currently being used to stabilize the secondary structure with a short amino acid sequence. For **E/K** hybrids, previous studies (unpublished data) have achieved **c** by introducing an unnatural amino acid (3-(2-naphthyl)-L-alanine), but not **b** (Fig. 1). Therefore, we attempted to apply **a** + **b** method to the **E/K** hybrids as well as the **Bim** peptides to form a more effective and smaller complex. These peptides were synthesized by peptide solid-phase synthesis and purified using RP-HPLC. Purified peptides were identified using MALDI-Tof-Mass. Secondary structure of the peptide was assessed from CD spectra and hybrid formation was assessed from fluorescence spectra.

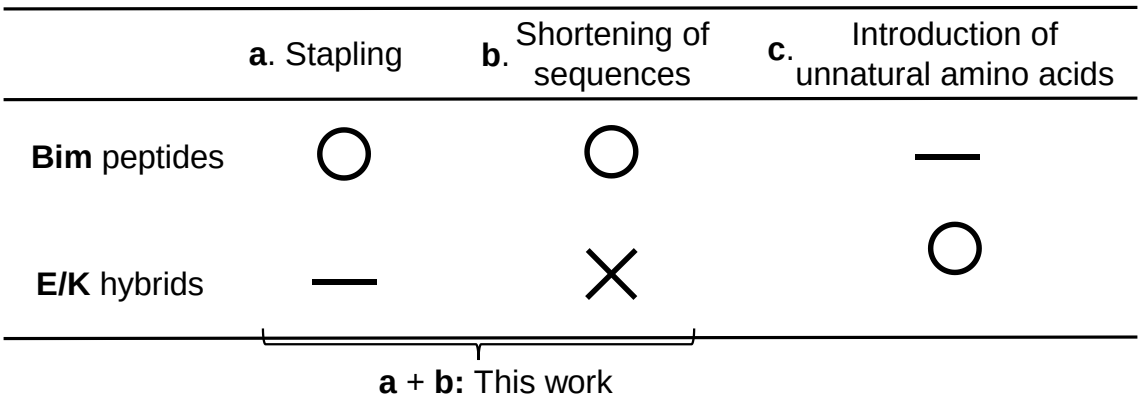

Fig. 1. Table summarizing the position of this study with respect to **Bim** peptides and **E/K** hybrids. Substitution of the amino acid units of the **Bim** peptide with Dap, Dab, Orn and Lys and stapling via these units showed stabilization of its secondary structure (α-helix). The N- and C-terminal amino acid units of the **Bim** peptide was successfully shortened while maintaining its ability to induce apoptosis. The interaction was enhanced by introducing non-natural amino acids(3-(2-naphthyl)-L-alanine) into the **E/K** hybrid.

Reference

[1]Zhou, S.; Watanabe, K.; Koide, S.; Kitamatsu, M.; Ohtsuki, T. Bioorg. Med. Chem. Lett. **2021**, *36*, 127811.