Synthesis and properties of α-helical peptides doubly-crosslinked with isophthalic acid-based crosslinking agents

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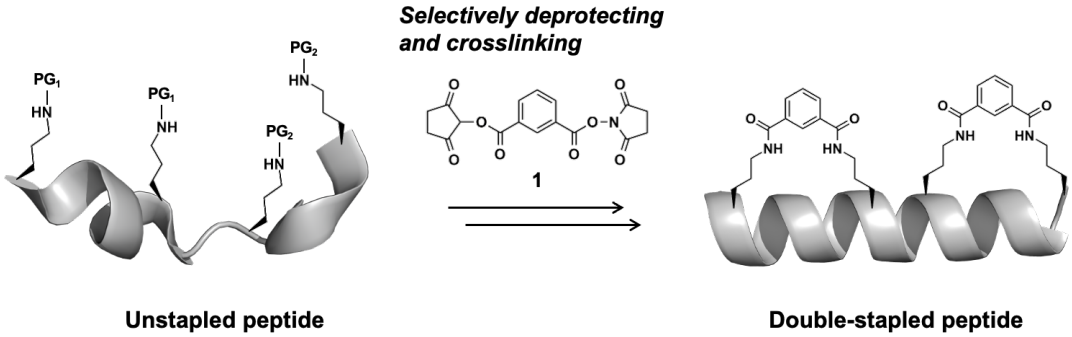
Protein-Protein Interactions (PPIs) play a crucial role in various biological functions. We have developed an isophthalic acid-based crosslinking agent **1** for creating α-helical peptides. In the native peptides, two ornithine residues were placed at *i* and *i*+7 positions as a stapling point for the crosslinking. The crosslinked peptides with **1** retained high α-helical contents, presenting a simple helix-reinforcing strategy only using naturally occurring amino acids. In addition, our stapled peptides having the binding domain sequence of an apoptosis-inducing protein showed higher proteolytic stability than that of the unstapled counterpart. The stapled peptides exhibited internalization into HeLa and HCT-116 cells and induced their apoptosis.[1)]

In this time, we planned to build up double-stapled peptides by using **1** in order to further increase the proteolytic stability. We picked up a sequence of the reported R1AD peptide that selectively binds to protein kinase A regulatory subunit 1α (PKA-RIα). The R1AD peptide was optimized to disrupt PPIs between PKA-RIα and A-kinase anchoring proteins (AKAPs). However, the peptide was easily cleaved by ubiquitous proteases. Single- and double-stapled R1AD peptides were prepared in high yields by means of conventional solid-phase peptide synthesis (SPPS) with selective deprotection of ornithine residues and subsequent crosslinking with **1**. The double-stapled peptide showed significantly high helicity and high affinity for the target protein PKA-RIα (K_d = 0.11 nM). Furthermore, the double-stapled peptide displayed high proteolytic stability and efficient intracellular uptake compared to those of the unstapled and the single-stapled ones.



1) M. Inouye et al. *ChemBioChem* 2014, *15*, 2571–2576; *Chem. Commun.* 2017, *53*, 12104–12107.

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