The 1,3-diyne linker as a tunable tool for peptide secondary structure stabilization

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Introduction

Protein-protein interactions (PPIs) control essential cellular processes and influence several biological functions.[1] However, despite their fundamental roles, PPIs are considered to be rather cumbersome targets for drug design due to their large and often flat contact surfaces, which cannot easily be addressed by small molecules. Nonetheless, the modulation of PPIs is important for the elucidation of biological processes and it represents a promising strategy towards next-generation therapeutics. One rational approach for the design of PPI inhibitors consists of a structure-based approach, wherein the topology of protein epitopes can serve as a starting point.[2] These epitopes are defined by the secondary structure of the underlying amino acid chain.[1] Among different successful strategies, the stabilization of α -helical peptide conformations can be accomplished by introducing covalent links between amino acid side chains at selected positions.[3] As a result, these ‘stapled’ peptides are often characterized by an improved binding affinity, metabolic stability and enhanced cell penetration, as compared to their linear peptide counterparts.[4,5] When aiming at α -helix mimetics, in particular, a macrocyclization involving the amino acids side chains at the i,i+4 or i,i+7 positions is often applied to bridge one or two helical turns, respectively.[1,6] Whereas a vast amount of work has been done on i,i+4 cyclizations [7], the current report focusses on constraints bridging two loops *via* an i,i+7 cyclization.

Results and Discussion

In light of the increasing interest for alkyne-alkyne coupling reactions in peptide chemistry,[8,9] our group verified whether O-propargylated serine residues were able to cross-link two helical turns in a i,i+7 fashion. Additionally, we evaluated the influence of the O-propargylated serines’ configuration on the α -helical stabilization by means of circular dichroism (CD).

In search for a suited model system, stapled peptide analogues able to inhibit MDM2 and MDMX proteins, important regulators of the transcriptional activity and stability of p53, were selected. In this context, Sawyer and co-workers designed an α -helical peptide (ATSP-7041) bearing an all-hydrocarbon staple, which proved to be a highly potent dual inhibitor of both MDM2 and MDMX.[10] We employed this 12-mer peptide as a model system and replaced the α -methyl- α -alkenyl amino acids in ATSP-7041 with O-propargylated serines for the evaluation of the 1,3-diyne linker as a rigid staple.

Previously, our group cyclized small peptidic macrocycles *via* a Glaser-Hay coupling of terminal alkynes.[8] We used the optimized coupling conditions to cyclize the envisaged linear peptide Ac-Leu-Thr-Phe-Ser(O-propargyl)-Ala-Tyr-Trp-Ala-Gln-Leu-Ser(O-propargyl)-Ser-NH₂ in solution, as well as on solid support. However, in both cases, the seemingly straightforward alkyne alkyne coupling failed to provide the cyclized peptide sequence. Therefore, the cyclization step was switched to a lactamisation, whereby the 1,3-diyne staple is introduced *via* insertion of a diyne-bearing dipeptide (4) by means of regular SPPS.

Scheme 1: Synthesis of dipeptide diyne building block 4.

Using 1 equivalent of 1 and 3 equivalents of 2 in the presence of $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$, NiCl_2 , Et_3N and pyridine in EtOH after 4 h at room temperature under an oxygen balloon, resulted in the formation of 3 in 55% isolated yield. Under these reaction conditions, homodimerization of terminal alkynes 1 and 2 was also observed, but both types of homodiyne were easily separated from heterodiyne 3 *via* silica gel chromatography in 6% and 17% yield, respectively. Simultaneous Boc- and tert-butyl deprotection by TFA treatment and subsequent Fmoc-protection led to the formation of dipeptide 4.

Dipeptide 4 was then used as a building block in regular Fmoc-SPPS on a Rink amide resin. After assembly of the first subsequence 5, saponification of the methyl ester on resin was performed using 10 equivalents of LiOH in DMF/ H_2O . In a next step, lactamization was achieved after 15 h by adding 3 equivalents of PyBOP and 6 equivalents of DIPEA in DMF, and followed by Alloc-deprotection with $\text{Pd}(\text{PPh}_3)_4$ and morpholine as nucleophilic scavenger. Finally, the final 3 amino acid residues were added, followed by acetylation of the terminal amine, cleavage from the resin and purification *via* preparative RP-HPLC, yielding cyclic peptide 7. To evaluate the influence of the configuration of the O-propargylated serine residues on the stabilization of helicity, 3 additional diyne dipeptides with different configurations (i.e., D-L, L-D and D-D) were synthesized in a similar fashion, to assess the ‘stapling efficiency’ of the cyclic peptides. The linear reference counterparts were also synthesized.

Scheme 2: Representative synthesis of macrocyclic peptide 7.

Next, the helical character of stapled peptides 7-10 was analyzed with circular dichroism (CD) spectroscopy. The peptides were dissolved in water and water/TFE mixtures with increasing concentrations of TFE to mimic a hydrophobic environment. Peptides (L,L)-7 and (D,L)-8 show a more pronounced helical character in $\text{H}_2\text{O}/\text{TFE}$ mixtures and TFE. On the contrary, peptides (L,D)-9 and (D,D)-10 display only a slightly helical conformation in $\text{H}_2\text{O}/\text{TFE}$ mixtures. Comparing the CD measurements of the stapled peptides 7 and 8 with their linear counterparts in $\text{H}_2\text{O}/\text{TFE}$ mixtures (50/50) shows that cyclic peptide 7 has less helical character than his linear counterpart, whereas stapled peptide 8 a similar helical profile has compared to the corresponding acyclic peptide.

Conclusion

In conclusion, we showed that the 1,3-diyne linker can be used as a rigid staple to stabilize short α -helical peptides by crosslinking residues in positions *i*, *i*+7. CD analysis confirmed the helical secondary structure of the prepared peptides. A full secondary structure characterization by NMR is envisaged to confirm the α -helical propensity of the macrocyclized peptides.

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