Design, synthesis and biological evaluation of stapled helical peptides targeting the myddosome

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Introduction

Protein-protein interactions (PPI) are involved in the regulation of a large variety of biological processes and their malfunctioning may lead to the development of diseases. These targets, considered as largely undruggable less than a decade ago, are receiving an increased interest in the search for novel therapeutic agents.[1] An important challenge originates from the extended contact surfaces of interaction. Peptides as small protein segments can cover larger surface areas as compared to small molecules, while three-dimensional stability can be preserved *via* chemical modifications, such as macrocyclization or helical constraints.[2-4]

The target of this study is the intracellular adapter protein MyD88 which is involved in the signaling pathways of the Toll-like receptor (TLRs) and Interleukin-1 receptor (IL-1), both playing a crucial role in the innate immune and inflammatory response. The MyD88 protein consists of two domains; a TIR domain that interacts with TLRs and IL-1R, and a death domain which multimerizes with Interleukin-1 receptor-associated kinases (IRAKs) to form the myddosome.[5] A previously obtained X-ray structure of this challenging PPI target shows a left-handed helical oligomer that consists of several MyD88 proteins, IRAK4 and IRAK2 death domains, resulting in highly complex interaction networks.[6] Three types of interfaces (type I-III) are revealed when the multimeric protein is unfolded along the helical axis. The type I interface, composed by a seven amino acid long helical epitope (-[E]YLEIR[Q]-) from MyD88, is particularly interesting as it shows several interaction points with IRAK4 (highlighted in bold). Moreover, a non-occupied hydrophobic pocket on the IRAK4 side is found in front of one of the MyD88 epitope side chains. Supported by molecular modeling, rationally designed stapled peptides were synthesized as helix mimetics of the type I epitope to be evaluated as potential inhibitors of this PPI.

Results and Discussion

Short stapled peptide analogues of the MyD88 type I interface epitope (-[E]YLEIR[Q]-) have been designed using an i,i+4 macrocyclization strategy to induce a helical conformation and potentially increase *in vivo* stability and selectivity. Linear peptide substrates were synthesized *via* SPPS on Fmoc-Rink aminomethyl polystyrene resin (0.1 mmol/reactor scale) using a Liberty Lite peptide synthesizer, or were assembled through manual SPPS. Different cyclization strategies were evaluated using the linear peptide sequences on solid-support, including copper(I)-catalyzed alkyne/azide cycloaddition (CuAAC), lactamisation and ring-closing metathesis (Scheme 1). Robust reaction conditions have been obtained for the different macrocyclizations strategies, resulting in a series of different stapled peptides of which the most relevant examples are depicted in Scheme 1.

Surface plasmon resonance (SPR), used to determine the binding of the (macrocyclized) peptides to IRAK4 protein, qualitatively identified multiple compounds with binding affinity to the IRAK4 domain, with KD values estimated between 100 to 200 μ M. Also, circular dichroism (CD) spectroscopy confirmed that macrocyclization successfully stabilizes alpha helicity in several of the short sequence peptides. By calculating the relative helicity of the different cyclic peptides[7], the highest helicity was observed for triazole macrocycle 2 (85%, Figure 1). According to the applied formula, this azide-alkyne cycloaddition product(2) showed a significant higher helicity compared to products 5-8 from lactamization and ring-closing metathesis cyclization strategies (helicity ranging from 42 to 58%). In attempts to further improve the potency and helicity of the cyclic CuAAC hit compound1 (Figure 1), the position of the staple was shifted to afford cyclic peptide 3, however, this was found to result in a loss of affinity compared to 1 which indicates the importance of the E1, R6 and Q7 residues for binding. With the same purpose, an Aib-scan (2-aminoisobutyric acid) was performed on the cyclic CuAAC hit compound1. This effort showed that the binding affinity could be preserved for analogue 4, although surprisingly a reduced helicity (58%) was calculated from the CD-spectrum compared to 1 (66%).

Scheme 1: Macrocyclization strategies via copper catalyzed-alkene/alkyne cycloaddition (CuAAC), lactamization and ring-closing metathesis (left) and prepared cyclic peptides (right)

Figure 1: Surface plasmon resonance (SPR) spectrum of peptide 1, injected for 60 seconds at 20 μ M (blue curve) and 200 μ M (brown curve) displaying a high binding affinity to IRAK4 (left) and Circular Dichroism (CD) spectrum of 2 which showed the highest alpha helicity (right)

Conclusion

Different macrocyclization chemistries have been optimized on the type I epitope of MyD88 to design stapled, helix stabilizing peptide mimetics. Surface plasmon resonance (SPR) data showed that ten staples peptides were good binders to the IRAK4 target. Moreover, circular dichroism analysis demonstrated that the used cyclization chemistries successfully conferred helicity to the very short sequence peptide mimetics, as compared to the linear counterparts.

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