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Peptoids for the Inhibition of Cytokines

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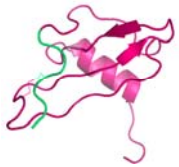
Abstract

Cytokines are small signaling proteins which play a key role in the human immune response. Chemotactic cytokines (chemokines) induce the directed migration of leukocytes. For several CXC-class chemokines, like Interleukin-8, the N-terminal “ELR”-motif is essential for receptor activation.[1,2] In the course of this work pentameric peptoids are synthesized which bind to the ELR-motif due to ionic interactions thereby preventing the binding of the chemokine to its receptor. The binding of the peptoids to the protein is tested by incubating the resin-bound peptoid with the fluorescently labelled N-terminal sequence of a chemokine. The fluorescence of each bead can be quantified using a microarray scanner. The hits are analyzed by mass spectrometry.

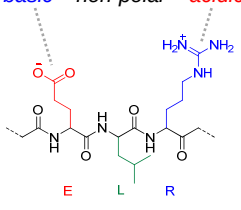
Synthesis

Peptoids are synthesized using the submonomer-method.[3] In order to bind the chemokine N-terminus the peptoid side chains must complement the charges of the ELR-motif giving rise to the following lead-structure for the peptoids:

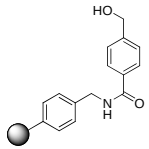
non-polar – basic – non-polar – acidic – non-polar



The structure of Interleukin-8, a chemokine of the CXC family



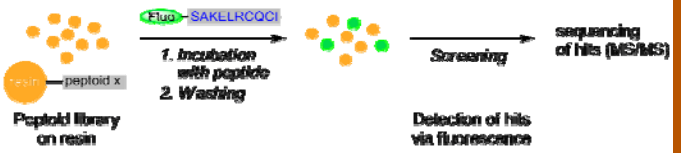
The ELR-motif at the N-terminus of CXC-chemokines



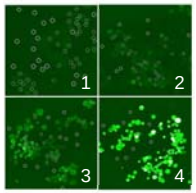
HMBA-AM resin, cleavage of peptoids with ammonia

HMBA-AM resin is used so that the side-chains can be deprotected without cleaving the peptoids from the resin.

Fluorescence-based binding assays



The resin-bound peptoids are incubated with a fluorescent model-peptide which represents the N-terminus of Interleukin-8.

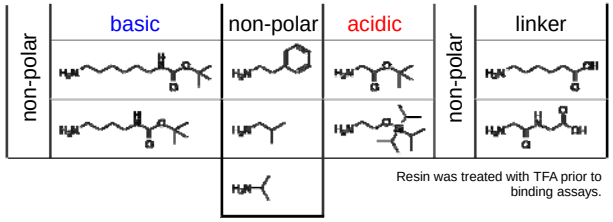


The fluorescence of individual beads is determined using a microarray scanner. The beads are transferred onto adhesive film and placed on glass slides. The fluorescence is measured at 532 nm.

1: resin without peptoid, 2, 3: peptoids without binding ability
4: peptoid (B7) with bound model-peptide (screening positive/hit)

Peptoid Analysis via Mass Spectrometry

A small library of pentameric peptoids has been synthesized to determine the applicability of mass spectrometry for the distinct identification of peptoids.
The Y- and B-series ions are typical for the peptoid fragmentation in MS/MS spectra.[4]

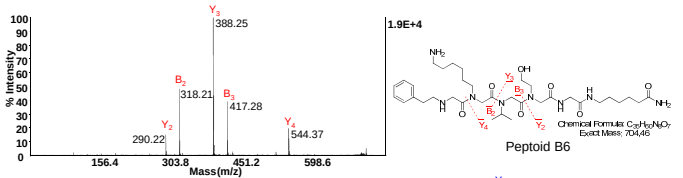


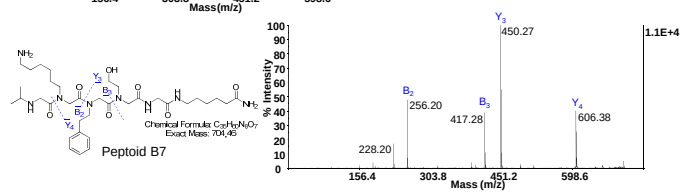
Resin was treated with TFA prior to binding assays.

Four amines of the pentameric sequence were gradually replaced by glycine to test if the residues have any effect on the expected Y- and B-fragmentation.

MALDI MS/MS fragmentation patterns

Peptoids that vary in sequence but not in their overall mass can be positively identified based on their Y- and B-fragments.





Summary and Outlook

- The identification of peptoids via mass spectrometry is possible.
- A first potential ligand was detected (peptoid B7) and the average bead-fluorescence was quantified with a microarray scanner.
- In the future various new submonomers will be used to create a greater diversity for screening assays.
- Larger peptoid libraries will be synthesized using the mix-and-split method. Hits from the peptide binding assay will be identified by MS/MS.
- Hits will be synthesized in larger scale for binding assays with full length chemokines and migration assays with leukocytes.
- The peptoid sequence will be increased to decamers to enhance the specificity of binding to different cytokines.

References

[1] C. A. Hébert, R. V. Vitangcol, J. B. Baker, *J Biol Chem* **1991**, *266* (28), 18989-18994.
[2] C. Bizzarri *et al.*, *Pharmacol Ther* **2006**, *112*, 139-149.
[3] R. Zuckermann *et al.*, *J Am Chem Soc* **1992**, *114*, 10646-10647.
[4] W. Heerma, C. Versluis, C. G. de Koster, *Rapid Comm Mass Spectrom* **1996**, *10*, 459-464.

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