

Peptoids for the Inhibition of Cytokines

Dorothea Helmer, Katja Schmitz

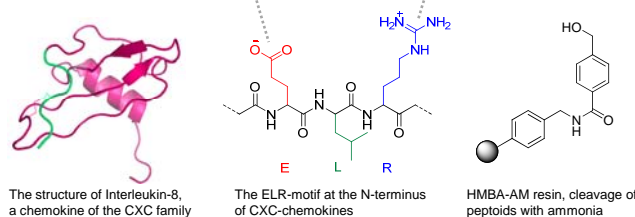
Abstract

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

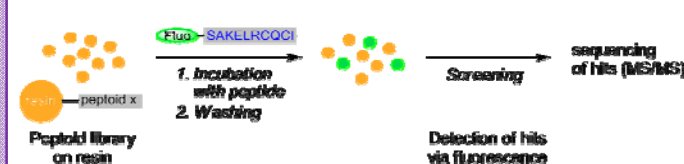
Peptoids are synthesized using the submonomer-method.³ In order to bind the chemokine N-terminus the peptoid side chains must complement the charges of the ELR-motif giving rise to the following lead-structure for the peptoids:

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

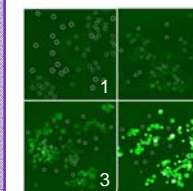


HMBAM resin is used so that the side-chains can be deprotected without cleaving the peptoids from the resin.

Fluorescence-based binding assays



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



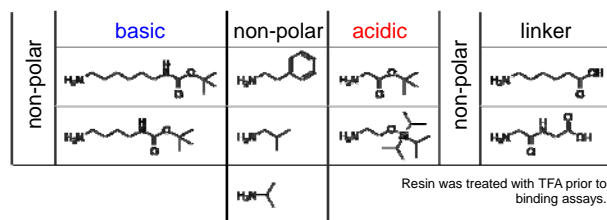
The fluorescence of individual beads is determined using a microarray scanner. The beads are transferred onto adhesive film and placed on glass slides. The fluorescence is measured at 532 nm.

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.

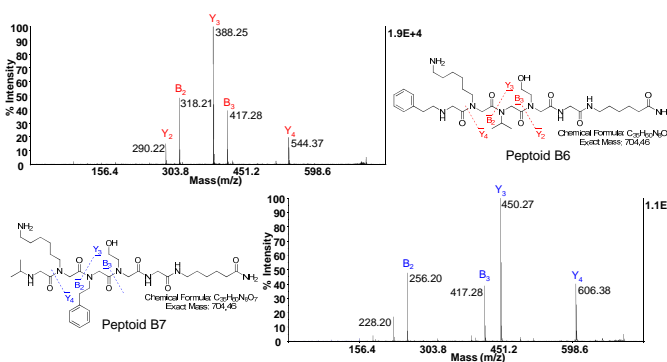
The Y- and B-series ions are typical for the peptoid fragmentation in MS/MS spectra.⁴



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.