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Expression and purification of recombinant chemokines for binding studies and cell migration assays

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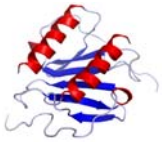
ABSTRACT

Chemokines are small soluble proteins which guide the migration of leukocytes. These proteins are produced by different cell types like epithelial cells and macrophages during an inflammation. Leukocytes which have the chemokine receptors on their surface follow the chemokine gradient so that they arrive at their destination point. Due to the influence of chemokines in inflammatory processes, autoimmune diseases, cancer and allergies, there is a large interest to manipulate or inhibit this immune reaction. For the use in binding studies with peptides or peptoids and cell migration assays large quantities of chemokines are required. The aim of this project is the expression and purification of human recombinant chemokines, e.g. Interleukin-8 (IL-8) from *E. coli*. The purified chemokines are used in activity studies and binding assays.

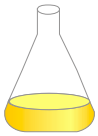


Interleukin-8

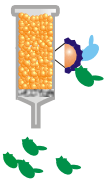
Stromal cell derived factor 1α

METHODS

- Expression of recombinant Interleukin-8 tag-free and with His-tag in *E. coli*
- Expression of other recombinant chemokines, e.g. His-tagged SDF-1α and Eotaxin-1
- Protein purification by various chromatographic methods
- Endotoxin removal and detection
- Cell migration- and binding assays using recombinant Interleukin-8



Expression of recombinant chemokines in *E. coli*



Purification by affinity chromatography

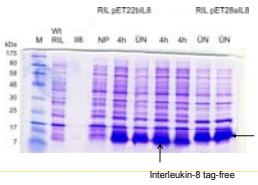
Binding assays with recombinant chemokines

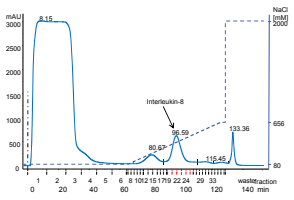
RESULTS

Expression of recombinant Interleukin-8 in *E.coli* (tag-free and with His-tag)

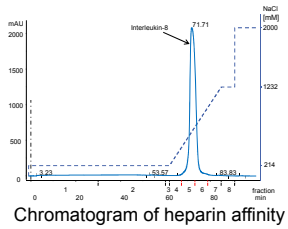
The purification steps of the tag-free recombinant chemokine are CIEX, affinity chromatography and gel filtration.

Expression of recombinant Interleukin-8 in *E. coli*





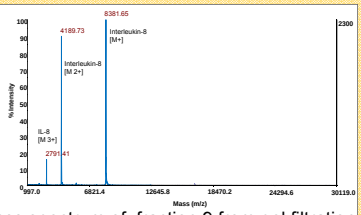
Chromatogram of CIEX



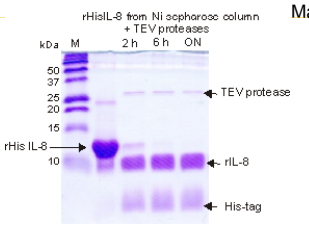
Chromatogram of heparin affinity chromatography

RESULTS

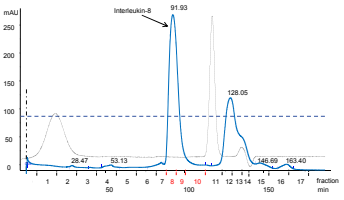
Purification steps of His-tagged IL-8 are affinity chromatography on a Ni-NTA- sepharose column, cleavage of the tag by TEV protease and gel filtration.



Mass spectrum of fraction 9 from gel filtration



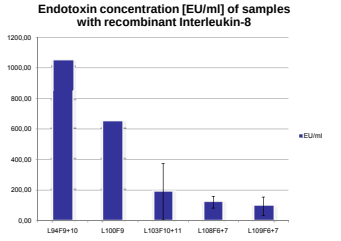
Recombinant HisIL-8 + TEV protease for tag removal



Chromatogram of the gel filtration after cleavage of the His-tag

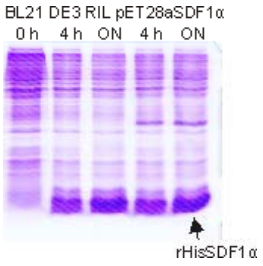
RECENT EXPERIMENTAL WORK

Endotoxin removal and detection from solutions with recombinant chemokines
Expression and purification of recombinant SDF-1α



L94F9+10 without endotoxin removal
L100F9 using detergent 1 in cell lysis buffer
L103F10+11 using detergent 2 and two cycles of phase separation
L108F6+7 using detergent 2 in cell lysis buffer
L109F6+7 using detergent 2 and three cycles of phase separation

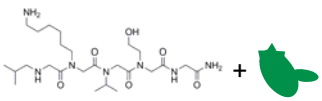
Endotoxin concentration from protein solutions after different strategies to remove endotoxin



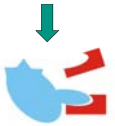
Expression of recombinant SDF1α with His-Tag

FUTURE WORK

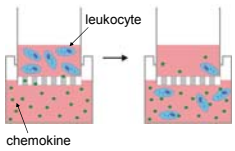
- Purification of other chemokines, e.g. SDF-1α
- Efficient removal of endotoxin from recombinant chemokines
- Testing of recombinant chemokines in binding assays with peptoids which specifically bind to IL-8 (see poster by Dorothea Helmer)
- Cell migration assays with rIL-8 and neutrophil granulocytes (see poster by Maria Braun)



Synthetic peptiods



Binding studies



Cell migration assay