Introduction

Recent studies reveal the key role of epidermal lipoxygenases (eLOX) from *Homo sapiens* and *Ambystoma mexicanum* (mexican axolotl) in wound healing and epidermis regeneration\(^1\). The epidermal lipoxygenase in mammals is involved in the regulation of keratinocyte differentiation. In the mexican salamander (axolotl) an epidermal lipoxygenase could be detected during limb development and in epidermal cells. Furthermore, it could be shown that human cells also respond to the epidermal lipoxygenases from the axolotl. Therefore, epidermal lipoxygenases offer a promising tool for clinical applications in wound healing within the multidisciplinary project “Biofabrication for NIFE”. Such alternative options are especially important in cases where conventional treatments fail.

Epidermal lipoxygenases as a new approach in wound healing: Heterologous production of human ALOXE3 in *E. coli*

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**Experimental procedure**

- Cloning ALOXE3 into suitable vector (e.g. pET28b) and verification
- Transformation and selection of *E. coli* production strain
- Optimization of soluble protein expression (e.g. temperature, inducer concentration)

**Results**

**A:** ALOXE3 could be cloned successfully into a pET28b vector. A polyhistidine-tag and a TEV protease recognition site upstream of the cloned gene can be utilized for subsequent purification.

**B:** Production of ALOXE3 in *E. coli* BL21 (DE3) led to insoluble protein fractions only. Different approaches for optimization of soluble protein production such as lower temperatures, different inducer concentrations and different media did not lead to a higher amount of soluble protein. Soluble protein production could be implemented by coexpression of certain chaperones using the commercial TaKaRa Chaperon Plasmid Set.

**General parameters found to work best in complex media:**
- OD for induction: 0,8
- Concentration of inducer (IPTG): 0,2 mM
- Cultivation temperature during protein expression: 20 °C

**A side note to chaperones:**
- Heterologous protein production in *E. coli* often leads to formation of inclusion bodies and/or degradation of the protein of interest by proteases
- Coexpression of chaperones can decrease these problems and lead to a correctly folded protein

**Plasmid**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Chaperones</th>
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<tbody>
<tr>
<td>pG-KJE8</td>
<td>DnaK, DnaJ, GroEL, GroES, DnaJ</td>
</tr>
<tr>
<td>pGp777</td>
<td>DnaK, DnaJ, GroEL</td>
</tr>
<tr>
<td>pKJE7</td>
<td>DnaK, DnaJ, GroEL</td>
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**Conclusions**

The human epidermal lipoxygenase ALOXE3 could be produced as a soluble protein in *E. coli*. In this case an approach using chaperone plasmids for coexpression did lead to the best results. Utilizing the polyhistidine-tag upstream of the gene, the produced protein can be purified and analyzed using different assays. Furthermore, a similar approach can be used for the heterologous production of the epidermal lipoxygenase AmbLOXe from the mexican axolotl.

References


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