Production of Purified Chondroitinase ABC II in use for Studies of Spinal Cord Nerve Regeneration

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Introduction

Figure 1. Overview of the experiment. Chondroitin sulfate proteoglycan (CSPG) after spinal cord injury forms a molecular barrier which restricts axonal regeneration and prevents functional recovery (Chau et al., 2003). It has been suggested that chondroitinase ABC (ChABC), a crude enzyme obtained from Proteus vulgaris, can digest the CS glycoforms. Therefore, the infusion of ChABC to the host side of the interface would aid axonal regrowth in severed nerves (Chau et al., 2003). There are two types of ChABC: type I and II. ChABC II’s digestion rate is quite low (Hamai et al, 1997). Protein modification is needed to maximize the digestion efficiency of ChABC II. Yet, we do not know its exact structure. We can identify its structure through the process of crystallization and X-ray crystallography. To do so, we will need a large concentration of ChABC II, in its purest form. The goal of this study is to develop methods of ChABC II purification and concentration, so crystallization and protein engineering can be done in the future.

Objectives

1) Produce a large quantity of chondroitinase ABC II (ChABC II)
2) Purify proteins
3) Concentration the purified ChABC II to 10mg/mL

Methods

1. **E. coli** was transfected with the gene for HIS tag and ChABC II
2. **E. coli** was grown in LB broth. Lysis buffer with protease inhibitors were added to the cultures, and the culture was sonicated.
3. Impure protein’s activity was measured with the spectrometer and concentration was calculated
4. Proteins were purified using a HIS select nickel affinity column. Fractions were collected, and protein concentration was measured using the Bradford assay.
5. Purified protein’s activity was measured with the spectrometer and concentration was calculated.
6. SDS polyacrylamide gel electrophoresis was performed.
7. Purified ChABC II was concentrated using an amicon filter.

Results

Figure 2. Bradford assay of ChABC II. The absorbance of each fraction was measured. Fractions 19-80, with the absorbance level of 0.3 or higher, were collected for analysis of enzyme activity and concentration.

<table>
<thead>
<tr>
<th>Fraction number</th>
<th>Abundance (A595)</th>
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<tbody>
<tr>
<td>20</td>
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<td>25</td>
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<td>35</td>
<td>0.4</td>
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<tr>
<td>40</td>
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<td>45</td>
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<tr>
<td>65</td>
<td>1.0</td>
</tr>
<tr>
<td>70</td>
<td>1.0</td>
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</table>

Figure 3. SDS Polyacrylamide gel electrophoresis with ChABC II. The second lane contains the unpurified proteins, and rest contains the purified. Results show that the purification was successful because 120kda is ChABC II’s molecular weight. It is evident that bands at fractions 40-65 have the most protein for those bands are the darkest.

Final protein concentration = 1.9 mg/ml
Fold of purification
Specific activity of unpurified proteins
specific activity of concentrated purified proteins
= [0.0402]/[0.0234] = 1.7

Conclusions

- We successfully purified ChABC II, as shown in figure 3.
- Final concentration of 1.9mg/mL was reached.
- Concentration of purified ChABC II was much lower than expected, because of the excess precipitation during the amicon process.
- The coagulation of undesired proteins might explain the presence of increased precipitation.
- In order for crystallization to occur, ChABC II needs to be further purified for a concentration of 10mg/μL is needed.
- Ion exchange column should be ran before the nickel-affinity column

References


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