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Enzyme purification from fermentation broth using membrane adsorber based centrifugal devices

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1. Introduction

The purification of biological active compounds such as proteins and enzymes from complex samples is a crucial step in proteomics and down-stream processing. Traditional chromatographic methods often require several steps resulting in time consuming and costly procedures. In contrast protein purification via membrane adsorber offers the advantage of fast and gently isolation due to their favourable pore structure allowing high flow rates without causing high back pressure. Mass transfer takes place through convection rather than diffusion and thus allowing a rapid performance. Therefore membrane adsorber can be used for a great number of different applications and are still gaining importance for the purification of biomolecules.

2. Penicillin-G-Amidase

In our work we showed the isolation of the enzyme penicillin-G-amidase (penicillin acylase) from the crude supernatant which was achieved using the anion exchange spin column within one step at a considerable purity. Penicillin acylase is an enzyme that catalyses the hydrolytic cleavage of penicillin G into 6-amino-penicillianic (6-APA) and phenyl acetic acid. This enzyme, found e. g. in *E. coli* or *Streptomyces* contains a 22 kDa and a 65 kDa subunit, which are processed from a 90 kDa precursor.

3. Material and Methods

The isolation of the enzyme was performed utilising a recently developed kit from VivaScience AG called SCOUT KIT. The two membrane adsorber based separation media within the kit are designed as centrifugal devices allowing to screen simultaneously several different loading and elution conditions with ion exchange matrices (cation and anion exchange) for the determination of the optimal purification conditions for the protein of interest in a complex sample. All materials required (separation units, buffers) are combined in a ready to use box. First, the binding capacity of Pen-G-Amidase (in *E. coli* lysate) on the cation and anion matrix with 4 different pH conditions (4, 6, 8, 9) was tested in 8 individual experiments.To avoid high salt conditions the cell lysate was diluted 1:10 in the 4 different buffers. After determination of the right binding conditions, the enzyme was purified via two different approaches.

4.1 Results

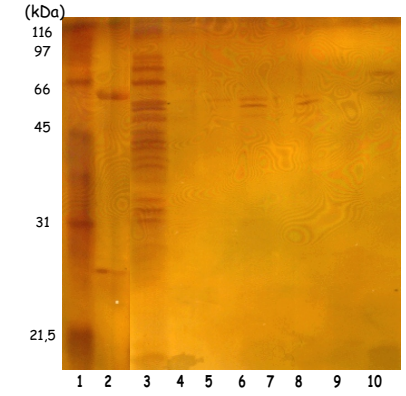

Fig 1: 12% SDS-Gel, silver stained. Sample was diluted in 25 mM Na_2Co_3-Buffer, pH 9 and loaded onto a Q spin column. The second wash step and the following elution steps were done in 25 mM KPP-Buffer, pH 6

1. Marker
2. Pen-G-Am.-Standard
3. Sample
4. Flow through
5. 1th Wash
6. 2nd Wash
7. 1th Elution (100 mM NaCl)
8. 2nd Elution (200 mM NaCl)
9. 3rd Elution (400 mM NaCl)
10. 4th Elution (1500 mM NaCl)

4.2 Results

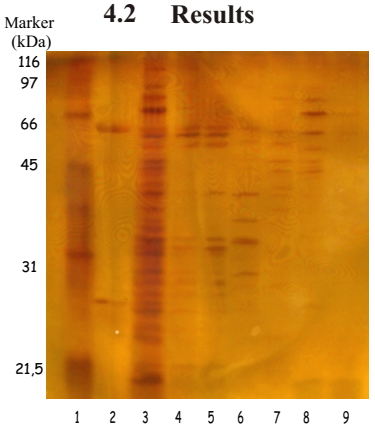

Fig 2: 12% SDS-Gel, silver stained. Sample was diluted in 25 mM KPP-Buffer, pH 6 and purified via a Q spin column (anoin exchanger)

1. Marker
2. Pen-G-Am.-Standard
3. Sample
4. Flow through
5. Wash
6. 1th Elution (100 mM NaCl)
7. 2nd Elution (200 mM NaCl)
8. 3rd Elution (400 mM NaCl)
9. 4th Elution (800 mM NaCl)

5. Discussion

In Figure 2 it was shown that almost all proteins of the lysate fraction were bound to the Q-membrane (anion-exchanger), except the penicillin acylase. The protein could easily be separated in the flow through by using an elution buffer with a pH of 6 (Fig 2, lane 4). The isoelectric point (PI) of the enzyme is in the range of 6-7, thus the charge of the protein is about zero in a buffer with a pH of 6. Consequently, Penicillin Acylase could not bind to the membrane and was efficiently purified in the flow through. In a buffer with a pH of 9 the whole protein fraction was bound onto the Q-membrane (Fig 1, lane 4). By using a washing and elution buffer with a pH of 6, the enzyme was partly purified in several steps (Fig 1, lane 5, 6, 8).

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