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One-step-purification of penicillin G amidase from cell lysate using ion-exchange membrane adsorbers

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Abstract

This study describes the purification of penicillin G amidase (PGA) by ion exchange membrane adsorbers in a one-step-process. Preliminary experiments with high-throughput screening devices in microliter scale (8-strip modules) were performed to find suitable purification strategy and appropriate ion exchange ligands as well as basic process conditions for binding and elution. Best purification results were achieved by strong cation-exchange (S-) membrane adsorbers loaded with 2 ml/min enzyme solution at pH 6.0 and eluted at pH 6.0 with 0.05 M NaCl, which led to a high yield of bound PGA (98%) without any visible remains of host cell proteins and with a residual enzyme activity of 80-85%. The binding of PGA to the S-membrane was further investigated in an upscaling to milliliter scale with LP15 modules and breakthrough curves were determined by varying the flow rates: the PGA-binding to S-membrane adsorbers is independent of the flow rate. Dynamic binding capacities were estimated to be 0.69 mg PGA/cm² (25.5 mg/ml) for 10% breakthrough respectively 0.95 mg/cm² (35.2 mg/ml) for 100% breakthrough. Finally, real cell lysate samples from *E.coli* culture containing PGA were processed under the found optimal conditions. Despite exceeded

loading PGA was isolated from this complex mixture successfully fourfold concentrated and with a purification factor of 101.3 and a resulting specific activity of 4.97 U/mg.

Highlights:

- Penicillin G amidase (PGA) was purified from cell lysate in a one-step-purification
- Ion-exchange (IEX) membrane adsorbers (MA) successfully used for the purification
- Direct capture IEX membrane adsorbers enabled a direct processing of cell lysate
- Direct capture MA yielded 98% PGA with min. 80% residual enzyme activity

Keywords: penicillin G amidase, membrane adsorber, ion-exchange chromatography

1. Introduction

The isolation and purification of any product from complex cell extracts is a crucial step in downstream processing and cumulates up to 80 % of the total costs of the process [1-3]. Chromatographic methods are of universal use, especially for the separation and purification of biomolecules as they distribute high affinity and process stability. Many downstream processes consist of at least two or more chromatographic steps to produce the pure biomolecule. But regarding the purification of low-concentrated biomolecules, as proteins and enzymes often are, conventional bed-packed chromatography is limited by low throughput and high pressure drop, as well as film and pore diffusion and flow rate dependent dynamic capacities [4-7]. This is very disadvantageous given that low product concentrations of proteins and enzymes in the harvest require fast processing of large volumes to keep the products conformation, accounting for nativity and activity of the biomolecules. Furthermore traditional chromatographic methods often require several steps resulting in lengthy procedures, which contradict also the need for fast processing in the downstream process. Thus there is a gap in downstream processing unit operations for low concentrated biomolecules with high affinity and large quantity throughput.

Membrane adsorption could be the method to bridge this gap and thus gained importance over the last years. The basic membrane adsorber consists of modified and microporous membranes with different ligands on the inner surface area. Due to their pore structure the mass transfer rate is not controlled by film and pore diffusion like in bead-based chromatography but primarily takes place through convection [3]. Thus, processing time and elution volume decrease tremendously. Therefore membrane adsorbers allow a rapid processing of low-concentrated products with high flow rates without causing high back

pressure. Furthermore constant binding capacities even at higher flow rates can be achieved. Another advantage of the membrane adsorption is to facilitate upscaling in comparison to bed-packed systems, because the membranes can be reeled up as a kitchen roll to spirally wound modules enabling large membrane surfaces in very small volumes.

Many papers deal with the use of membrane adsorption for purification and isolation of proteins [26-28] and antibodies or to remove DNA, host cell proteins, endotoxins und viruses respectively [2, 7-14]. Membrane adsorbers employ the same strong anion and cation exchange ligands, quarternary ammonium (Q) respectively sulfonic acid (S), as standard ion exchange chromatography columns. In many downstream processes ion exchange chromatography is one of the last steps - the polishing step. Especially the anion exchange chromatography (AEX) has been validated to provide significant removal of present viruses [10, 14].

Penicillin G amidase (PGA), the model enzyme for the isolation and purification in this work, has been extensively investigated already and been isolated and purified with several techniques in multiple-step processes [15]. PGA (EC 3.5.1.11) catalyzes the enzymatic hydrolysis of penicillin molecules to 6-aminopenicillanic acid (APA) and phenylacetic acid (PAA). The latter is a key intermediate in the synthesis of semi-synthetic antibiotics. On this account PGA is one of the most widely used enzymes applied in the industrial production of semisynthetic ß-lactam antibiotics like penicillin V und ampicillin [15-20]. Most of the papers dealing with the purification of PGA focus on hydrophobic interaction and ion-exchange chromatography or a combination of these methods [1, 15, 16, 20, 21]. Other used chromatographic methods are hydroxyapatite, affinity or pseudo-affinity chromatography [15, 18, 19]. Additionally streptomycin or ammonia sulfate precipitation was investigated [15, 21, 22]. All these PGA purifications are multiple-step processes resulting in long process times.

In this work we investigated the possibility to isolate and purify PGA in only one chromatographic step based on ion exchange membrane adsorption. First a parallel screening of basic binding conditions was performed using microliter scale ion-exchanges membranes in so-called 8-strip adsorber modules for 96-well arrangement. Then the process was upscaled to milliliter scale (LP 15 modules) and analyzed in regard to binding capacity, protein recovery and residual enzymatic activity of the eluted enzyme. The whole process was finally adapted to cell lysate from *E.coli* cultivation containing PGA to evaluate the feasibility as a downstream unit operation for industrial use.

2. Experimental

2.1 Chemicals

Chemicals for buffer preparation, protein assay, enzymatic activity assay, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) and silver staining of the gels were purchased from Sigma-Aldrich, Steinheim, Germany or from Carl Roth, Karlsruhe, Germany. All chemicals were of *per analysis* quality. All buffers and protein solutions were prepared in demineralized water (Arium, Sartorius AG, Göttingen, Germany). Pure penicillin G amidase standard was purchased from ASA Enzyme, Wolfenbüttel, Germany. 6-Nitro-3-phenylacetamidobenzoic acid (NIPAB) for enzymatic activity assay were prepared according to Zhang et al, 1986 [23] in the Institute of Organic Chemistry, Leibniz University Hannover, Hannover, Germany.

2.2 Membrane adsorber

High-throughput experiments were performed with quarternary ammonium (Sartobind© Q) anion exchange and sulfonic acid (Sartobind© S) cation exchange membrane adsorbers (96-Well plate containing 8-strips: 3 membrane layers with a total adsorption area of 0.7 cm^2 per well, 300 μL maximum sample volume per centrifuge run with 500·*g*, Sartorius Stedium Biotech GmbH, Göttingen, Figure 1 left).

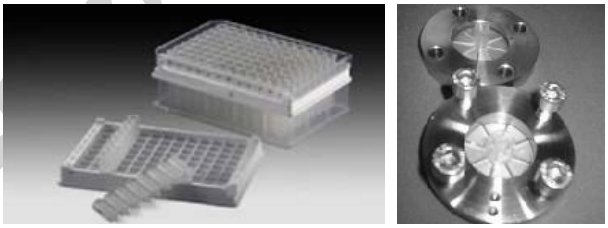

Figure 1: 8-strip membrane adsorbers placed on 96-well plates are used in a centrifuge (left),
LP-15 Membrane adsorber test systems can be used for analyzing performance in an up-scale to milliliters (right)

The membranes consist of stabilized reinforced cellulose with a pore size of 3-5 μm. The 8-strips were placed on 96-well plates and processed in a centrifuge with a swing out rotor (Megafuge 1.0RS, Heraeus Instruments, Hanau, Germany).

Dynamic binding capacity and protein recovery were determined by using the LP 15 adsorber module. These are adsorber test systems which are developed for analyzing membrane

adsorber performance in a scale of milliliters. They consist of a screw-in module of steel where three membrane stamps, each with a diameter of 5 cm², were inserted in the module.

2.3 Cultivation procedure

The recombinant *E. coli* strain 5KpHM12 [24] for penicillin G amidase production (PGA, MW 70 kDa, pI 8.5) was cultivated in shaking flasks with 100 mL of LB medium (10 g/L yeast extract, 10 g/L peptone, 5 g/L NaCl) with 10 mg/L thiamine, 1 g/L glucose, 100 mg/L glycine and 10 mg/L tetracycline. The cultures were left to grow for ca. 8 h up to an optical density OD_{600} of 8.0-9.0 at 37 °C and 130 rpm. The cells were harvested by centrifugation at 3,345·*g* for 30 min (4 °C), resuspended in sodium phosphate buffer pH 7.5 and disrupted by sonication (four times 30 s each with 1 min break, 80 W, 0 °C). Subsequently, the cell debris was removed by centrifugation at 17,000·*g* for 20 min.

2.4 Binding and elution of penicillin G amidase in high-throughput screening

For the enzyme binding anion and cation exchangers were used at different pH values. Used binding buffers were: 25 mM sodium acetate buffer, pH 4 and pH 5, 25 mM potassium phosphate buffer, pH 6 and pH 7, 25 mM tris/HCl buffer, pH 7 and 25 mM $NaHCO_3$ buffer pH 9 and pH 10. The cell lysate with PGA was adjusted to the appropriate pH value by 1:5 dilution of cell lysate in the respective binding buffer. For elution, 50, 100, 200, 400, 600, 800, and 1,000 mM NaCl were added to the binding buffers.

First the membranes were equilibrated twice with appropriate binding buffer. Afterwards the cell lysate was loaded thrice on the membrane. The bound enzyme was eluted with the elution buffers in an elution sequence with increasing ionic strength. For all steps 300 µL maximum sample volume per centrifuge run with 500·*g* for 3 min were used.

2.5 Determination of dynamic binding capacity

UV detection for enzyme breakthrough can only be used for penicillin G amidase standards but not for all lysate samples**.** Other UV active compounds influence the analysis. Thus dynamic capacity was determined under optimal conditions using pure PGA standard. During the loading step, no enzyme is present in the effluent until the dynamic binding capacity of the membrane is reached. Dynamic binding capacity at 10 % breakthrough was defined as the ratio of enzyme concentration in effluent to enzyme concentration in the loading aliquots $C/C_0 = 0.1$. Here C is the concentration of PGA in the effluent and C_0 is the concentration of PGA in the loading solution. Accordingly, dynamic binding capacity 100 % was defined as

the ratio C/C_0 = 1.0. Effluent was collected in 2 mL fractions during the whole chromatographic process. The protein concentration in each fraction was determined by UV absorbance at 280 nm. For all these experiments Vivapure 5.4 membranes (Sartorius Stedium Biotech GmbH, Göttingen) which are in contrast to 8-strips not placed on 96-well plates but processed directly in a centrifuge with 500·*g* and a swing out rotor were used. 500 µL maximum sample volume per centrifuge run were taken. The membranes were equilibrated with 6 mL 25 mM potassium phosphate buffer, pH 6 with a flow rate of 1 mL/min. After equilibration the membrane adsorber was loaded with a 2 mg/mL enzyme solution. The elution of the bound penicillin G amidase was achieved using a 25 mM potassium phosphate buffer, pH 6 with 50 mM NaCl.

2.6 SDS-PAGE

The purity of the obtained fractions was verified by standard denaturing SDS–PAGE on 12 % gels under reducing conditions according to the method of Laemmli using an electrophoresis unit from BIO-RAD (München, Germany). A protein ladder (PageRuler from Fermentas, St. Leon-Rot, Germany) was used as molecular size marker. The protein bands were visualized via silver staining.

2.7 Protein assay

Protein concentrations in solutions were established either by the Bradford assay or by measurement of absorbance at 280 nm using a multiskan spectrum microplate spectrophotometer (Thermo Electron Corporation, Vantaa, Finland). Bovine serum albumin (BSA) was used as standard.

2.8 Enzymatic activity

The enzymatic activity of PGA was determined photometrically using 6-nitro-3-phenylacetamidobenzoic acid (NIPAB) as substrate. 6 mM NIPAB was dissolved in 50 mM sodium phosphate buffer, pH 7.5. 900 µL buffer and 500 µL NIPAB-solution were added in a 1 cm cuvette. The reaction was started by adding 100 µL of the enzyme solution and the production of 6-nitro-3-aminobenzoic acid was detected at 405 nm. The activity of PGA was calculated by the following expression:

$$A = \frac{\Delta E}{t \cdot v \cdot 2{,}99}\left[\frac{\mu mol}{\min \cdot ml}\right] = \left[\frac{U}{ml}\right]$$

ΔE = difference of extinction

t = time [min]

v = volume of enzyme solution [mL]

The factor 2.99 comprises cuvette length, molar amount of sample and extinction coefficient for the product 5-amino-2-nitrobenzoic acid. One unit of enzyme activity (U) is defined as the amount of enzyme which catalyzes the formation of 1 µmol of 6-nitro-3-aminobenzoic acid per minute. The purification factor was calculated as the quotient of specific PGA-activity before and after purification.

3. Results and Discussion

For the isolation and purification of penicillin G amidase from cell lysate by ion exchange chromatography two principally different strategies are possible and described in literature. First the so called "negative" separation of the enzyme by depletion of all host cell proteins through binding them unspecifically to an ion exchange material, thus collecting the PGA in the flow-through [25] and second the specific binding of PGA itself to an ion exchange material, keeping all host cell proteins (HCP) in the flow-through and subsequent elution of the bound enzyme [9]. Both strategies were tested in the described microliter scale high-throughput screening (see chapter 2.2) with cell lysate to identify best binding material and evaluate basic process conditions.

3.1 Purification of penicillin G amidase by anion exchange

The cell lysate from *E.coli* 5KpHM12 shake-flask cultivation after cell lysis and centrifugation was loaded onto a Q-membrane (strong AEX membrane with quarternary ammonium ligands) at different pH values to investigate the pH dependency on the PGA purification. The isoelectric point of PGA is at pH 8.5. At pH values above the pI, the PGA is net negative charged and can interact with the positively charged quaternary ammonium

ligands. Thus, the higher the loading pH, the more PGA should adsorb onto the Q-membrane; this could actually be found.

The cell lysate was adjusted to pH 6.0; 7.0; 8.0; 9.0; 10.0. Higher pH values were not applied due to denaturing effects of strong alkaline pH. At pH 6.0 - 8.0 (lower than the enzyme pI) no significant amount of PGA was bound to the membrane, but also most HCP retained in the flow-through. Thus the strategy to deplete host cell proteins from the target enzyme by binding them to the ion exchange membrane was not successful.

As expected the PGA bound at pH values above the pI to the membrane. At pH 9.0 a significant amount of PGA was bound and at pH 10.0, the total PGA was trapped. Unfortunately, next to the enzyme all other host cell proteins of the cell lysate also bound to the membrane, showing no significant separation effect. The unspecific binding of HCP furthermore decreased binding capacity of PGA onto the Q-membrane significantly (data not shown).

Anyway, the most promising strategy with AEX membranes proved to be the specific binding of the enzyme at high pH and separate elution. Therefore optimal elution conditions were screened by loading the enzyme at pH 10.0 onto the membrane. Subsequently the particular wells of the 8-strip were eluted with different pH values (pH 4.0 - 10.0) and ion concentrations from 0.2 - 1.0 M NaCl.

Elution of the PGA was successful for all tested pH-values, but ionic strength had to be raised simultaneously with increasing pH to elute the bound PGA. As shown in Figure 2 no pH value/ionic strength-combination was found able to elute the pure enzyme. At every elution condition the PGA was eluted together with host cell proteins from the membrane. Figure 2 shows the results of SDS-PAGE for different elution fractions.

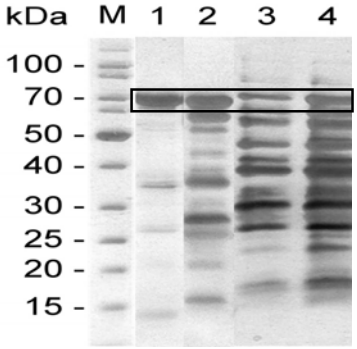

Figure 2. SDS-PAGE: Elution of PGA from a Q-membrane. Lane M: molecular weight marker, lane 1: elution at pH 6 with 200 mM NaCl, lane 2: elution at pH 7 with 200 mM NaCl, lane 3: elution at pH 8 with 400 mM NaCl, lane 4: elution at pH 9 with 400 mM NaCl.

Finally, the best results for the purification of PGA with the strong AEX membrane were obtained by loading the cell lysate at pH 10 onto the membrane and eluting the bound enzyme at pH 6 with a NaCl concentration of 0.2 M. Fig. 2, lane 1 shows the purification result at these conditions with still visible impurities from HCP. Thus, the strong AEX membrane was considered as not applicable for a fast one-step-purification process for PGA.

3.2 Purification of penicillin G amidase by cation exchange

The screenings for the purification of PGA were also carried out with cation exchange material (Sartobind© - S membrane, strong cation exchange (CEX) membrane). The S-membrane contains sulfonic acid ligands on their inner membrane surface. At pH lower than the pI of PGA (< pH8.5), the enzyme is net positive charged and can interact with the negatively charged sulfonic acid groups. Corresponding to this no ionic interactions will take place at pH values higher than the pI, but a depletion of host cell proteins might be possible.

Consistent with the results of the PGA purification by Q-membrane, in the screenings no significant separation effect was observed through unspecific binding of HCP at high pH (data not shown). Thus, only specific binding of the enzyme was investigated in more detail (binding pH lower than enzyme pI).

The cell lysate was diluted with the appropriate binding buffers to pH 5.0 - 8.0. At pH 5.0 the total PGA amount as well as all HCP bound to the membrane. At pH > 5.0 most of the HCP were detected in the flow-through, but with the rising pH also increasing enzyme amounts were recovered in the flow-through; maximum enzyme adsorption took place at pH 5.0 (data not shown).

Based on this fact, the PGA-containing cell lysate was loaded at pH 5.0 onto the S-membrane for screening of elution conditions. These were tested as before under variation of pH (pH 3.0 - 8.0) and ionic strength from 0.05 - 1.0 M NaCl.

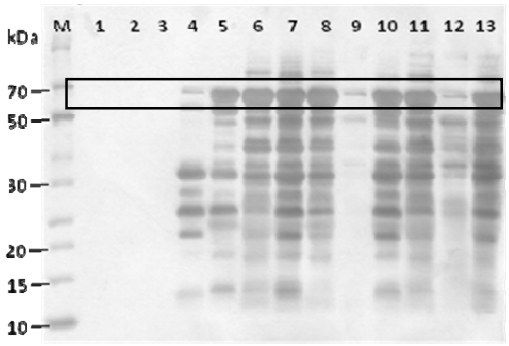

Figure 3. SDS-PAGE: Elution of PGA from S-membrane, loaded at pH 5. Lane M: molecular weight marker, lane 1, 2: flow-through fractions, lane 3: wash fraction. Elution at pH 5: lane 4: 100 mM NaCl, lane 5: 150 mM NaCl, lane 6: 200 mM NaCl. Elution at pH 6: lane 7: 100 mM NaCl, lane 8: 150 mM NaCl, lane 9: 200 mM NaCl. Elution at pH 7: lane 10: 100 mM NaCl, lane 11: 150 mM NaCl, lane 12: 200 mM NaCl. Lane 13: cell lysate.

Figure 3 shows the results of SDS-PAGE for different elution fractions (10 µL sample). Lane 1 and 2 show the flow-through during the loading of cell lysate, lane 3 shows the wash fraction. As described before, neither PGA nor other proteins of the cell lysate could be detected in these samples. Thus, at this loading pH all HCP adsorbed on the S-membrane along with the PGA, which is undesired, but generally pH 5.0 proved to be suitable to bind the target enzyme quantitatively.

In the other lanes of the SDS-PAGE in Figure 3 different elution fractions with pH- and ionic strength variation are plotted. It can be seen, that no separate elution of the PGA could be achieved after loading at pH 5.0. And it was found the higher the pH value of the elution buffer the lower the ion concentration needed to elute most of the proteins as well as the PGA. Therefore, in a further testing of the S-membrane the PGA loading-pH was adjusted to pH 6.0 and the optimal elution conditions were screened as above. It was found that elution of significant amounts of PGA was achieved at pH 6.0-8.0 and low ion concentration of 0.05-0.1 M NaCl (see Fig. 4). At pH values above pH 6.0 also other bound HCP were eluted. Different elution fractions are illustrated in Figure 4.

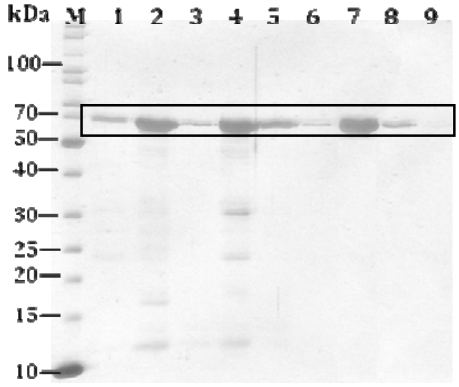

Figure 4. SDS-PAGE: Elution of PGA from S-membrane, loaded at pH 6. Lane M: molecular weight marker, lane 1: elution at pH 8 with 50 mM NaCl, lane 2: elution at pH 8 with 100 mM NaCl, lane 3: elution at pH 8 with 200 mM NaCl, lane 4: elution at pH 7 with 50 mM NaCl, lane 5: elution at pH 7 with 100 mM NaCl, lane 6: elution at pH 7 with 200 mM NaCl, lane 7: elution at pH 6 with 50 mM NaCl, lane 8: elution at pH 6 with 100 mM NaCl, lane 9: elution at pH 6 with 200 mM NaCl.

The best results for the purification of PGA with the strong CEX membrane were obtained after loading at pH 6.0 and elution at pH 6.0 with 0.05 M NaCl. With these settings it was possible to isolate pure PGA from cell lysate in a one-step-purification with a strong cation exchange membrane (S-membrane), yielding 97% of the bound PGA. The screening revealed the suitable basic conditions for the one-step-purification process which was upscaled to milliliter scale (LP15 adsorber modules) in the following to determine dynamic binding capacities, and residual enzyme activities; these experiments were carried out with standard protein samples. For further experiments in milliliter scale regarding the purification yield and process stability of the membrane adsorbers again cell lysate was used.

3.3 Determinations of dynamic binding capacity and enzyme activity

Determination of dynamic binding capacity in milliliter scale (LP15 adsorber modules) was carried out at a FPLC with UV detection using the S-membrane (15cm^2 membrane surface area) and pure PGA standard according to chapter 2.5. Optimal loading and elution conditions as determined by high-throughput screening (see above) were applied. Different breakthrough curves under variation of loading flow rates were determined. Experiments were performed with flow rates in the range of 0.2 - 5.0 mL/min. In Figure 5 an exemplary breakthrough curve (flow rate 1.0 ml/min) is shown.

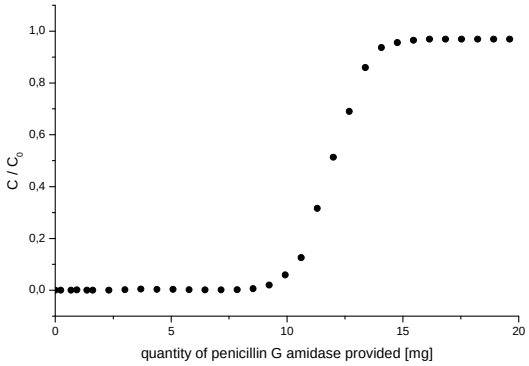

Figure 5: Purification of PGA with S-membrane, breakthrough curve at 1.0 ml/min flow rate.

The variation of the loading flow rate showed no decrease of the dynamic binding capacity as Figure 6 shows. The dynamic binding capacity at 10 % breakthrough for the adsorption of PGA on the S-membrane was 0.69 mg/cm² (25.5mg/ml), whereas the dynamic binding capacity 100 % was 0.95 mg/cm² (35,2 mg/ml). It was possible to elute 98 % of the bound PGA from the membrane with the optimized conditions from high-throughput screening. The residual enzymatic activity of the eluted PGA was 80-85 % (see Fig. 7). The results showed no dependency on the flow rate, proving the proposed independency of membrane adsorbers from diffusion processes.

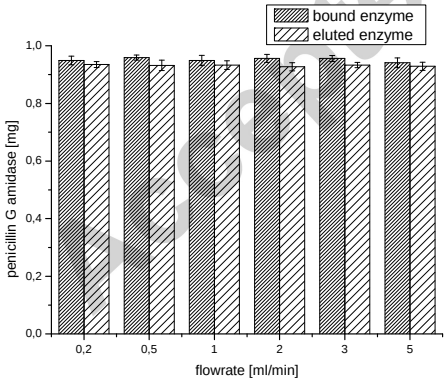

Figure 6: Effect of loading flow rate on the dynamic binding capacity.

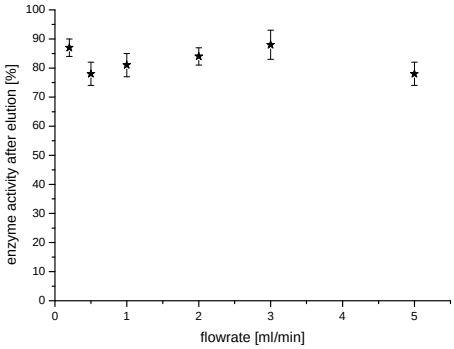

Figure 7: Residual PGA activity after purification

The determined basic separation conditions by high-throughput screening and the determined results in milliliter scale with standard protein solution were then adapted in a final step to a

one-step-purification process of PGA from real cell lysate from *E.coli* 5KpHM12 culture by LP15-milliliter scale CEX membrane adsorbers. The corresponding FPLC-run is displayed in Figure 8A, also showing the collected 2ml-fraction borders of all relevant process fractions.
The protein content of all relevant process fractions as shown in Figure 8A was estimated qualitatively by SDS-PAGE with sensitive silver staining as shown in Figure 8B.

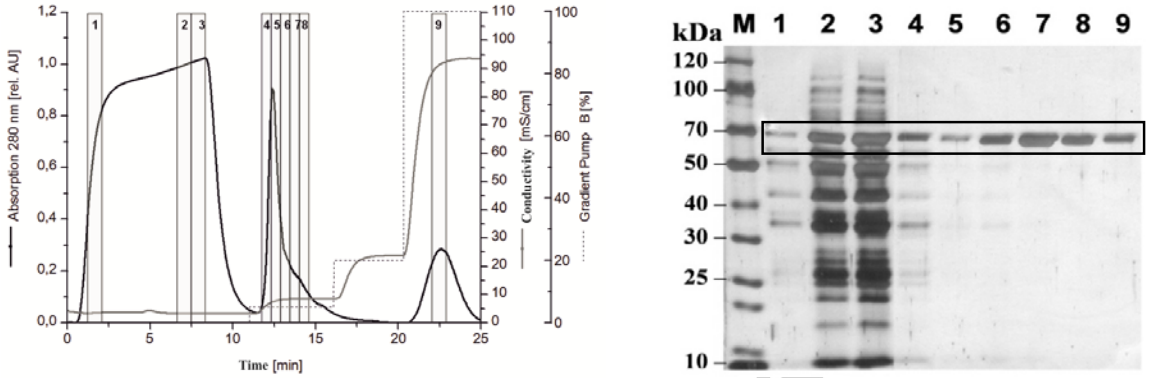

Figure 8A: FPLC-run of the one-step-purification process of PGA from real cell lysate from *E.coli* 5KpHM12 culture by LP15-milliliter scale CEX membrane adsorbers (collected fractions are indicated by numbered rectangles)
Figure 8B: Corresponding SDS-PAGE of the purification of PGA from cell lysate, S-membrane, loaded at pH 6 and eluted at pH 6 with 50 mM NaCl. Lane M: molecular weight marker, lane 1-3: flow-through fractions, lane 4: wash fraction, lane 5-9: elution at pH 6 with 50 mM NaCl.(12 % gel with silver staining)

The gel shows different fractions of the purification process as flow-through fractions (lane 1-3), wash fraction (lane 4) and elution fractions (lane 5-9). The PGA is visible in the circled area around 70KDa. The total protein concentration in the cell lysate was 72.96 mg/ml, comprising all HCP and PGA with a PGA-activity of 0.05U/mg. It can be seen that the PGA-binding capacity is exceeded, because large amounts of PGA are lost in the flow-through, indicating a far too small membrane surface area offered. Anyway, the binding of PGA is weak as seen from the visible band in the wash fraction but sufficient, for the PGA elutes fourfold concentrated nearly free of any host cell proteins from the membrane. The determined purification factor was 101.3 (specific activity 4.97U/mg) and the high specific activity furthermore shows that the membrane adsorption can serve as a suitable technique for fast and efficient downstream processing of PGA.

4. Conclusions

PGA-isolation and purification was successfully carried out in a one-step-purification process with ion exchange membrane adsorbers. Preliminary experiments with high-throughput screening devices (8-strip modules) indicated the suitable purification strategy and revealed appropriate ion exchange ligands (S-membrane adsorbers) as well as basic process conditions. Best purification was achieved by S-membrane adsorbers loaded at pH 6.0 and eluted at pH 6.0 with 0.05 M NaCl, which led to a high yield of bound PGA (98%) without any visible remains of host cell proteins and high residual enzyme activity of 80-85%. The binding of PGA to the S-membrane was investigated then more detailed and breakthrough curves were determined for varying flow rates; these showed independency of PGA-binding to S-membrane adsorbers of the flow rate, which proves that membrane adsorber ligands are reached by any target molecule through convective flow rather than through diffusion. Dynamic binding capacities were estimated to be 0.69 mg/cm² (25.5 mg/ml) PGA for 10% breakthrough respectively 0.95 mg/cm² (35.2 mg/ml) for 100% breakthrough. Finally, real cell lysate samples from *E.coli* 5KpHM12 culture containing PGA were processed under the found optimal conditions. Despite exceeded loading PGA was isolated from this complex mixture fourfold concentrated with a purification factor of 101.3 (4.97U/mg).

Thus, compared to different publications where the purification processes consist of multiple purification steps [17, 18, 22], it was possible to purify the PGA with a strong cation-exchange membrane in only one fast chromatographic step, thus facilitating the downstream processing significantly by decrease of unit operations, leading to a reduction of used chemicals and wastes as well as process time.

5. Acknowledgement

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