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Human growth hormone (hGH) purification from CHO-cell culture supernatant

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INTRODUCTION

The purification of biological active compounds such as human growth hormone from crude cell culture samples is a crucial step in down stream processing and proteomic research. Traditional chromatographic methods are based on packed columns and are associated with time consuming procedures and high back pressure. A large percentage of the adsorptive surface is located inside the pores of the particles and is only accessible by slow molecular diffusion.
In contrast, macroporous membranes and monoliths allow high flow rates without causing high back pressure. There is no mass transfer restriction, the mobile phase is forced to through the large pores of the chromatographic medium. Consequently, mass transfer takes place through convection rather than diffusion. As a result a high resolution can be achieved in a fast and gentle process, which means a benefit for the biological activity of sensitive products.

CELL CULTURE

Dihydrogenase reductase (dhfr)-deficient CHO cells transfected with a plasmid containing the hGH and dhfr gene were used for production of hGH. The cells were cultivated in ProCHO4-CDM supplemented with 4 mM L-glutamine and 0.1 µM MTX at 37°C, 5% CO_2 in spinner flasks. Cells were separated from the supernatant by centrifugation (400 g, 3 min) and the supernatant was concentrated via crossflow filtration (Vivaflow 50, 10 kDa MWCO).

MEMBRANE ADSORBER BASED CHROMATOGRAPHY

The purification of hGH via membrane adsorbers was performed utilizing Vivawell 8-strips in combination with a centrifuge. First a screening for the best purification conditions was performed using cation and anion exchanger membranes at 4 different pH conditions (pH 4.5, 6.0, 7.5, 9.0) in 8 individual experiments (fig. 1). To avoid high salt concentrations and to adjust the appropriate pH, the samples were diluted in the respective binding buffers. Two elution steps were carried out with salt concentrations of 300 and 600 mM NaCl. The best results were achieved using a cation exchanger membrane at pH 4.5.(25 mM NaAc, fig. 2).

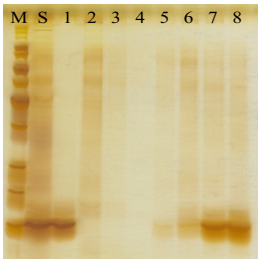


FIGURE 1.
15% SDS-Gel, silver stained. Screening for optimal purification conditions via Vivawell S 8-strip (cation exchanger) and Vivawell Q 8-strip (anion exchanger).

M: Marker ,
S: sample,
1: S membrane, pH4.5
2: S membrane, pH 6.0
3: S membrane, pH 7.5
4: S membrane, pH 9.0
1: Q membrane, pH4.5
2: Q membrane, pH 6.0
3: Q membrane, pH 7.5
4: Q membrane, pH 9.0

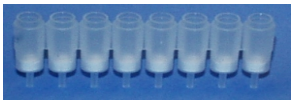
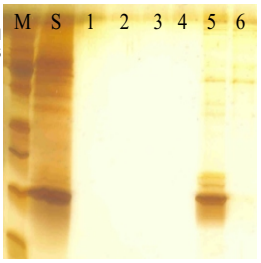


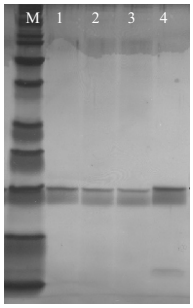
FIGURE 2
15% SDS-Gel, silver stained. Sample was diluted in 25 mM NaAc, pH 4.5 and purified via Vivawell S 8-strip (cation exchanger).

M: Marker ,
S: sample,
1: flow through,
2-4: wash fractions,
5: 1th Elution (300 mM NaCl),
6: 2nd Elution (600 mM NaCl)



MONOLYTIC DISK BASED CHROMATOGRAPHY

The purification of hGH via monolithic disks was performed utilizing epoxy activated CIM (Convective Interaction Media, BIA Separations). The disk was incubated in a anti-hGH solution (5mg/ml) in 50 mM Na_2CO_3 (pH 9.3), the immobilisation procedure was performed at 30°C for 16 h. The residual epoxy groups were quenched with 1 M ethanolamine. As a result 0.9 mg anti-hGH were bound on the affinity disk. The purification of hGH from cell culture supernatant was performed via HPLC (flow rate: 2 ml/min) in PBS (pH 7.0). To desorb unspecifically bound proteins, the disk was washed with 2 M NaCl, elution of hGH was achived with HCl, pH 2.0 (fig. 3).



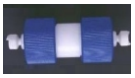


FIGURE 3
15% SDS-Gel, silver stained. Examination of different loading buffers via CIM disk. Elution was performed with HCl, pH 2.0.

M: Marker
1: 100 mM PBS, 150 mM NaCl
2: 200 mM PBS, 150 mM NaCl
3: 10 mM PBS, 150 mM NaCl
4: hGH standard

CONCLUSIONS

The purification of hGH via Vivawell 8-strips with cation exchanger membranes results in a considerable purity within one step (fig. 2, lane 5). More over, the device allows a highly parallel screening for the optimal purification conditions in a decent amount of time.
The affinity chromatography utilizing CIM disks succeeded in a purification within one step with high purity. Due to the fast procedure, the device is also applicable for screening procedures. Another advantage of this approach is the possibility to reutilize the disks over a long period of time.
In summary both devices have shown to be suitable tools for the fast purification of hGH from CHO cell culture supernatant.

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The Vivawell 8-strips were donated by Vivascience (Hannover, Germany).
Epoxy activated CIM disks were provided by BIA separations (Ljubljana, Slovenia).