Zentrum Angewandte Chemie
Institut für Technische Chemie

Institut für Technische Chemie, Callinstr. 3, 30167 Hannover

Universität Hannover

Human growth hormone (hGH) purification from CHO-cell supernatant using membrane adsorber based centrifugal devices

Johanna Walter[1], Kirstin Suck[1], Alexander Tappe[1], Cornelia Kasper[1] ,Robert Zeidler[2],Thomas Scheper[1]

[1]Institute for Technical Chemistry,
Callinstr. 3, 30167 Hannover, Germany

[2] Vivascience AG, Feodor-Lynen-Str. 21,
30625 Hannover, Germany

1 Introduction

The purification of proteins from complex cell culture samples is a crucial step in the proteomic research. 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 gentle isolation. Here, mass transfer takes place through convection rather than diffusion. Due to their pore structure membrane adsorber enable high flow rates without high back pressure.

2 human growth hormone (hGH)

The human growth hormone is produced by the pituitary gland and is responsible for the growth process of human beings. HGH consists of a single polypeptide chain with a weight of 21,5 kDa. Since microsomnia is one of the main consequences when lacking hGH, the protein is of pharmaceutical significance.

3 Experimental part

The isolation of the human growth hormone from a chinese hamster ovary (CHO) cell culture supernatant has been performed using the SCOUTING-KIT developed by Vivascience AG. Here, the membrane adsorbers are designed as centrifugal devices. Due to this, the devices allow to screen several loading and elution conditions with cation and anion exchanger matrixes in parallel, in order to determine the optimal purification conditions for the protein of interest. In 8 individual experiments the screening for the optimal binding and elution conditions has been performed at 4 different pH conditions (4.5, 6.0, 7.5, 9.0). To avoid high salt concentrations the samples has been diluted 1:5 in the respective binding buffers. The loading step has been carried out three times, to utilize the membranes capacity. Three elution steps with an increasing salt concentration from 300 to 900 mM sodium chloride have been performed. The best results have been achieved using an cation exchanger membrane at pH 4.5. In order to detect the hGH, the diluted sample, the flow through, wash fraction and the eluates have been analysed by SDS-PAGE. To examine the purification effect of the method the experiment has also been performed with a cell culture supernatant to witch 2 % human serum has been added.

4.1 Results

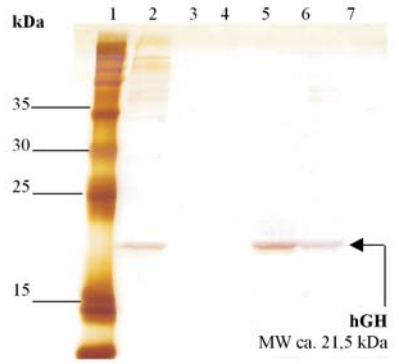

Fig 1: 15% SDS-Gel, double stained. Sample was diluted in 25 mM NaAc, pH 4,5 and purified via a Vivapure S Mini spin column (cation exchanger).

1. Marker
2. Sample
3. Flow through
4. Wash fraction
5. 1th Elution (300 mM NaCl)
6. 2nd Elution (600 mM NaCl)
7. 3rd Elution (900 mM NaCl)

4.2 Results

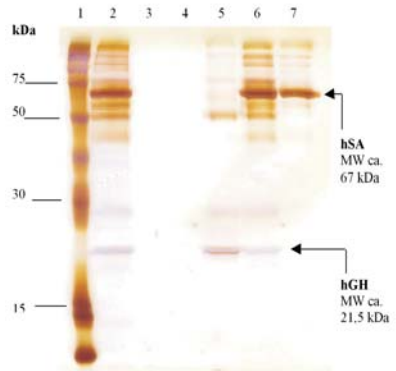

Fig 2: 15% SDS-Gel, double stained. Sample was diluted in 25 mM NaAc, pH 4,5 and purified via a Vivapure S Mini spin column (cation exchanger).

1. Marker
2. Sample
3. Flow through
4. Wash fraction
5. 1th Elution (300 mM NaCl)
6. 2nd Elution (600 mM NaCl)
7. 3rd Elution (900 mM NaCl)

5 Discussion

According to figure 1 the hGH has been bound to the S-Membrane at pH 4.5. In the first elution (line 5) the SDS-PAGE shows the hGH without any visible impurities. Due to the repeated loading step the hGH concentration in this fraction is higher than in the original sample. In the second elution (line 6) there are also smaller quantities of hGH, but there are still contaminants. Under the same conditions it was also possible to seperate hGH and hSA, although their isoelectric points have nearly similar values (hGH: 5.0; hSA: 5.2). According to figure 2 there is the bulk of the hGH without hSA in the first elution (line 5) but there are still some serum proteins this fraction.In summary the cation exchanger membrane has shown to be a suitable tool for the purification of hGH from this special cell supernatant.

VIVASCIENCE