Effects of Cell Culture Conditions on the Titer and Purity of Recombinant TNFR-Fc Expressed in CHO Cells

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Abstract

Cell culture conditions can influence the titer and quality of recombinant proteins expressed by CHO cells. TNFR-Fc fusion protein is a therapeutic protein expressed in CHO cells. Cell culture conditions not only affect the titer of TNFR-Fc, but also influence the purity, that is percentage of TNFR-Fc active dimer form. In this study, we aim to examine the effect of CHO cell culture conditions on the expression level and purity of TNFR-Fc fusion protein. Recombinant CHO cells expressing TNFR-Fc fusion protein were cultured under different conditions including feeding strategies, culture time and culture scales. The TNFR-Fc fusion protein was purified by protein A affinity chromatography. And the purity of dimer was analyzed using size exclusion chromatography. The titer of TNFR-Fc fusion protein increased gradually, while the purity decreased as the cultivation time extended. Compared to batch cultures, cell culture feeding medium mix1 on days 2, 4, and 6 showed higher expression level and lower purity(percentage of dimer). The expression level and purity of TNFR-Fc was stable when the culture scales enlarged from 50ml to 1, 5 and 30 L scales. These results indicated that medium feeding strategies, culture time and culture scales impacts the titer and purity of TNFR-Fc fusion protein during cell culture.

Keywords

TNFR-Fc Fusion Protein; CHO Cells; Purity; Multimer.

1. Introduction

Recombinant therapeutic antibodies are major part of today's pharmaceutical industry. Manufacturing of recombinant protein products using mammalian cell culture technology has made significant progress in the last 2 decades [1]. Chinese Hamster Ovarycell line (CHO) was the most used host cell because of their ability to produce proteins with similar post-translational modifications to that of an innate human protein [2]. In the manufacture of recombinant monoclonal antibodies, not only the titer, but also the quality of antibody are very important indicators.

The titer of proteins is the one of the most important factors in bioprocess optimization [3-5]. Studies have shown that the culture conditions such as temperature, feeding of nutrients, and culture time have a significant impact on the expression level [6]. For example, culturing at temperature lower than 37℃, or nutrients feeding can prolong the cell growth cycle and promote the titer of the target protein [7-11], however, the quality of recombinant proteins will be change with the prolonged cultivation time. Moreover, during manufacturing, formulation, and storage, the proteins undergo a variety of degradations, aggregations and et al , even for antibodies which are relatively stable [12]. Protein aggregates, one of the most common quality challangesin the preparation of high concentration antibodies, have been proposed to cause adverse effects, such as reduced efficacy and immunological reactions. It was found that different cell culture conditions could affect the level of protein aggregates [13] in cultures. For example, temperature, pH, agitation, addition of cell culture additives such as valproic acid (VPA) were identified as main critical factors influencing protein aggregation during cell culture [13,14]. The longer the protein was held in the cell culture medium at elevated temperature, the greater the amount of aggregates observed [15]. In fact, up to 30% of

aggregates has been reported for some mAbs in mammalian cell cultures [16], thus aggregation of protein is another factor to be studied in cell culture conditions optimization.

TNFR-Fc is a fusion protein by linking the extracellular domain of human soluble p75 TNF receptor with human IgG1 Fc fragment, which is an effective drug used to treat rheumatoid arthritis [17] and ankylosing spondylitis [18] and other chronic inflammatory diseases. TNFR-Fc has similar structure with monoclonal antibody and works as a dimer conjuncted with Fc fragments. However, during thecell cuture , the generation of multimers, monomers or degradation products of TNFR-Fc reduced the purity of the dimer forms, which increases the difficulty of subsequent purication. In this paper, we studied the influence of cell culture conditions such as culture time ,feeding on titer and purity of TNFR-Fc (percentage of dimers) in CHO cells.

2. Methods

2.1 Cell culture method

The recombinant CHO cells to express TNFR-Fc were inoculated at a cell concentration of 5×105 cells/mL serum-free Procho5 medium (LONZA, Switzerland), then were cultured at 37℃ at a stirring speed of 75-90 rpm (round per minute). On day 4, the temperature was shifted to 31℃, and the culture was supplemented with feeding medium every other day until harvest. The supernatants of CHO cuture was collected when the viability dropped below 40 %, and the expression and purity of TNFR-Fc fusion protein were analyzed.

2.2 TNFR-Fc fusion protein purification and titer analysis

After centrifuged at 4000 rpm/min for 20 minutes, the supernatants of CHO cell culture was collected and purified with Protein A chromatography. The supernatant was filtered through a 0.22 μm filter (Millipore, Billerica, MA) prior to applying onto the MabSelect ProteinA column, which equilibrated with 20 mM PB(pH 7.2) for 25 min at a flow rate of 5mL/min. Bound antibodies were eluted with 100 mM Glycine buffer(pH3.3) at 5 mL/min. The protein concentrations of the eluted fractions were then measured using the NanodropTM 2000/200c spectrophotometer.

2.3 Analysis of TNFR-Fc purity

Purity of TNFR-Fc (percentage Dimer forms) was analyzed using size-exclusion chromatography (SEC). SEC was performed using an Ultimate 3000 HPLC system with a TSK G3000SWXL column, 7.8 mm×300 mm (TOSOH, Japan). 20 ul sample were applied to the column which equilibrated with 0.5M NaCl, 20mmol/L PB, pH7.2, at a flow rate of 0.5 mL/min and monitored by a UV detector at 280 nm. The level of dimer forms was determined by the relative percentage of the peak areas.

Table 1. Different conditions of feeding

Group	Time of feeding	Medium
control	/	/
1	day2,4,6	mix1
2	day3,6	mix1
3	day2,4,6	mix1+GLN
4	day2,4,6	mix2
5	day3,6	mix2
6	day2,4,6	mix2+GLN
7	day3,6	mix3

3. Results

3.1 The influence of feeding on the titer and purity of TNFR-Fc

CHO cells were inoculated at 5×105 cells/mL in ProCHO (5) media, then fed with different supplements according Table 1, using cells cultured without supplement as control. After cultured at 37℃ for 72 hours, CHO cells were transferred to 31℃ in 50ml TubeSpin.

CHO cells were cultured in media supplemented with different feeds, and the culture was harvested when the viability became lower than 40%. As shown in Figure 1,the titer of TNFR-Fc produced by cells grown in media supplemented with Mix1 on day 2, 4, and 6 was the highest than other groups, which is about 220mg/L. While the control group had the highest purity compared with those fed-batch groups, which was about 95%, but lower titer of only about 140 mg/L.

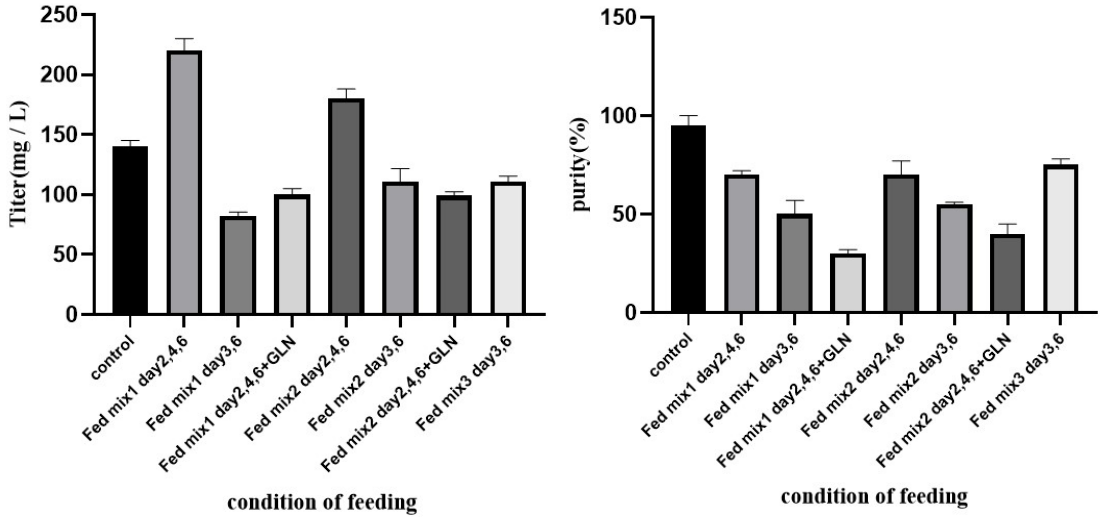

Figure 1. Titer and purity of TNFR-Fc expressed by CHO cells under different fed-batch culture conditions

3.2 The influence of culture time on the titer and purity of TNFR-Fc

Recombinant CHO cells were cultured at 37℃ for 72h and shifted to 31℃ in a 5L and bioreactor, cultures were sampled on day6, 7, 8, 9. As shown in Figure 2, the titer of TNFR-Fc fusion protein increased with the culture time,which increased from 195 mg/L on 6 days to 295 mg/L on 9 days. However, the purity of the TNFR-Fc fusion protein, that is, the percentage of active dimer, decreased as the cultivation time extended, decreased from 80% on 6 days to 65% on 9 days.

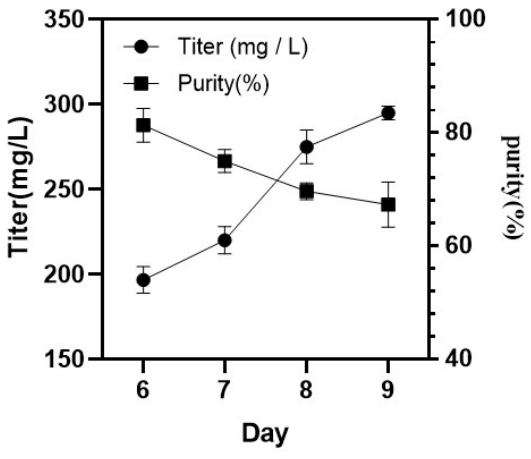

Figure 2. Titer and purity of TNFR-Fc expressed by CHO cells under different culture time

3.3 The influence of culture scales on the titer and purity of TNFR-Fc

CHO cells were cultured in different bioreactors scales from 50ml TubeSpin, 1L flask, to 5L and 30L bioreactors. After culturing at 37℃ for 72h, shift to 31℃ to continue cultivation. cultures were harvested once the viability dropped below 40 %. As shown in Figure 3, CHO cells cultured in 50ml TubeSpin had the highest titer of TNFR-Fc fusion protein that reached up to 370mg/L, and cultured in 30L bioreactor had the lowest that was about 275mg/L. When CHO cells cultured in 50ml TubeSpin, the purity of the TNFR-Fc fusion protein, that is, the percentage of active dimer, is 85%, which was similar with that in 1L shake flask, 5L, 30L bioreactor, respectively.

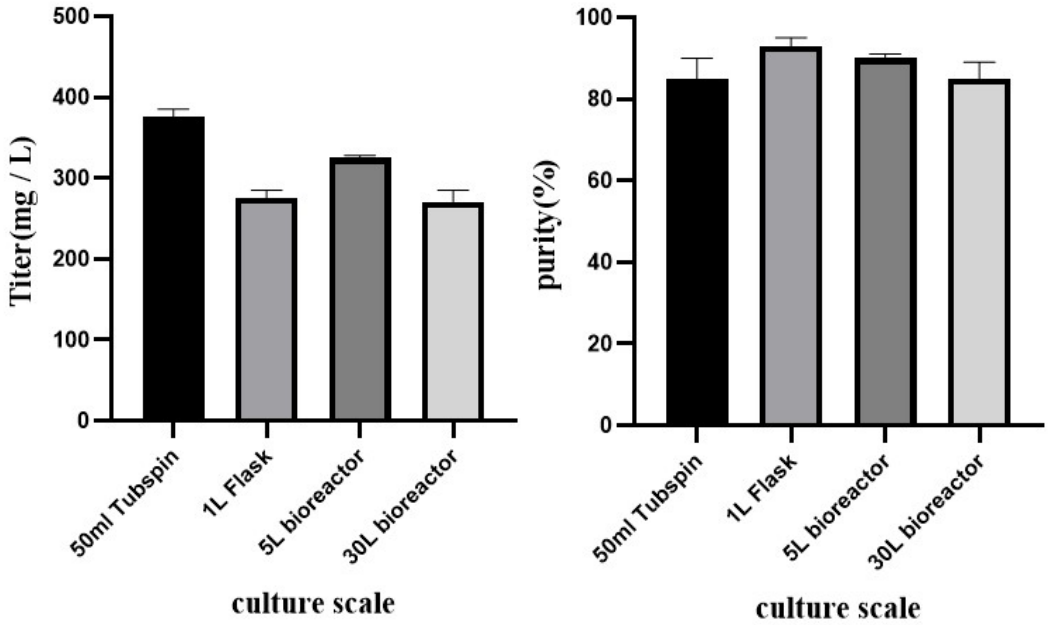

Figure 3. Titer and purity of TNFR-Fc expressed by CHO cells under different culture scales

4. Discussion

Due to the costs and complexities associated with the commercial production of mAbs, there is a strong demand to efficiently and economically deliver consistently high quality drug substance[19]. Increases in protein production have been attributed to bioprocess optimization of media, cell culture conditions, and enhanced feeding strategies[19]. In fed-batch culture, periodic delivery of appropriate feeds provides sufficient nutrients to support cell growth and metabolism in prolonged culture life[20]. However, accumulation of cellular by-products may inhibited cell growth, and increased multimer production or degradation,which will reduced the biological activity of protein drugs, and even cause the body's immune response[21-23].

Protein aggregation is a common phenomenon during protein manufacture. The TNFR-Fc obtained by one-step purification contained multimers except for the active form of dimer, and dimer and multimer could not be separated by Protein A affinity chromatography. Optimization of the cell culture conditions to increase the purity (percentage of dimer) and titer of TNFR-Fc during the cell culture process can reduce the production of multimers thus make antibody purification simple and achieve a high cost-benefit ratio.

Paradoxically, the best cell culture process for high titer of recombinant proteins usually is not the best conditions for high quality of it. In our study, theextend culture time could increase thetiter of TNFR-Fc, but decrease the purity, that is, the percentage of active dimer. Feeding supplemental media Mix1 on day 2, 4, and 6 could obtainthe highest titer of TNFR-Fc with purity of about 70%, while the culture without feeding could get the highest purity but lower titer . Thus the compromised strategy for cell cultre should be chosen.

Acknowledgements

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