Dimeric TNFR-Fc Decreasing in the Later Time of CHO Cell Culture

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Abstract

Recombinant human Tumor necrosis factor receptor-Fc (TNFR-Fc) is a recombination protein which is expressed in Chinese hamster ovary (CHO) cells. And the dimeric TNFR-Fc is desirable. In order to detect the component of culture production, we collected the cultural medium of recombinant CHO cell at 37℃. Then used Protein A column to purify the crude sample followed by detecting the percentage of dimeric TNFR-Fc fusion protein through SEC-HPLC and SDS-PAGE. And TNFR-Fc productivity was measured by ELISA. During the later period of CHO cell culture, CHO cell viability went down which meant cell living condition becoming unsuitable. Although the productivity of TNFR-Fc continued to increase, the percentage of dimeric TNFR-Fc decreased at the same time. After protein A column affinity purification, the production contained three parts, namely polymeric and dimeric TNFR-Fc as well as TNFR-Fc digestion.

Keywords

Productivity; Polymeric TNFR-Fc; Dimeric TNFR-Fc; CHO Cell.

1. Introduction

Tumor necrosis factor receptor-Fc (TNFR-Fc) is a dimeric recombination protein which is made of two extracellular fragments of tumor necrosis factor 2 conjugated with Fc of type 1 human immunoglobulin. Dimeric TNFR-Fc is conjugated through Fc fragment and has a higher affinity than monomer [1-3].

Chinese hamster ovary (CHO) cells have been broadly adapted to produce recombinant protein due to keeping its native configuration and easy purification [4].

There have been many papers on how to improve the productivity of TNFR-Fc [5-11]. And the quality of TNFR-Fc is also rather important which must be fully considered during the producing process. The quality control of TNFR-Fc includes a lot of requirements, such as molecular weight, specific activity, purity, isoelectric point [12]. Because impurity of recombinant biomedicine may lead to immune response [13], the purity of TNFR-Fc is an especially vital criterion and must be limited to an acceptable level.

In this study, we found the productivity of TNFR-Fc increased as culture time was prolonged. However, the percentage of dimeric TNFR-Fc decreased rapidly at the same time which inferred the quality of TNFR-Fc became worse. In order to exclude the impurity of TNFR-Fc effectively, we detected the component by SDS-PAGE after purifying culture medium with protein A column..

2. Materials and Methods

2.1 Cell Line and Cell Culture

Stable recombinant CHO cells were obtained by transfecting pxlg6-TNFR-Fc plasmid into CHO-DG-44 screened with puromycin. Serum-free Procho5 medium was purchased from LONZA and 4 mM glutamine, 0.68 mg/L hypoxanthine as well as 0.194 mg/L thymine were added. 2 L

recombinant CHO cells were cultivated in 5 L bioreactor (Biostat B Plus, Germany) under 37 ℃ with 40% O_2 and stirring 251 r/min.

2.2 ELISA

Cell suspension was taken from bioreactor every day. The sample was centrifugated at 1000 rpm for 10 min, the supernatant was stored at -20 ℃. Trypan blue stain was used to identify the viable cells and cell count was performed by Count Star (purchased from Shanghai Ruiyu Biotech Co. Ltd).

TNFR-Fc concentration was quantified by ELISA. Goat anti-human IgG (purchased from KPL) was coated to 96-well plates for overnight. Appropriately diluted standard TNFR-Fc and samples with BSA were added to wells and treated with goat anti-human IgG（H＋L）/AP (purchased from abcam). Finally, the substrate was added into wells and the absorption is determined at the wavelength of 450 nm.

2.3 Determination of TNFR-Fc Aggregation

TSKgel G3000 SWXL (Tosoh, Japan) sepharose gel column was used to determine the percentage of dimeric TNFR-Fc after affinity purification. Flowing phase was 0.5 mol/L Nacl, 20 mM PB, pH 7.2 with 0.5 mL/min flowing rate and 50 μL sample injection under room temperature. The integral was performed on chromatogram to calculate the proportion of dimeric TNFR-Fc.

3. Results

3.1 Growth of CHO Cell

CHO cell density and viability are shown in figure 1. Under the condition of 37 ℃, cells grew rapidly and cell density reached to 10^7 cells/mL on the fourth day and cell viability was above 90%. Then cell density began to decrease sharply. On tenth day, cell density reached to 3.0×10^6 cells/mL with 50.94% cell viability (Fig. 1A and 1B).

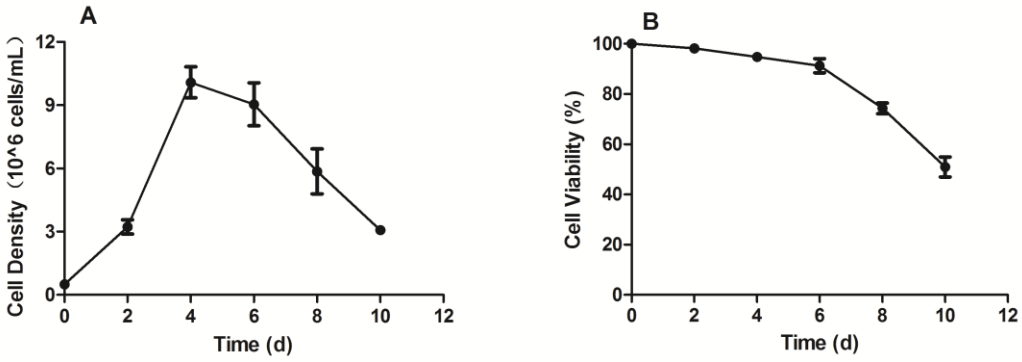

Fig. 1 CHO cell growth

Cell density. B.Cell viability. Culture was stopped when cell viability was under 50%.

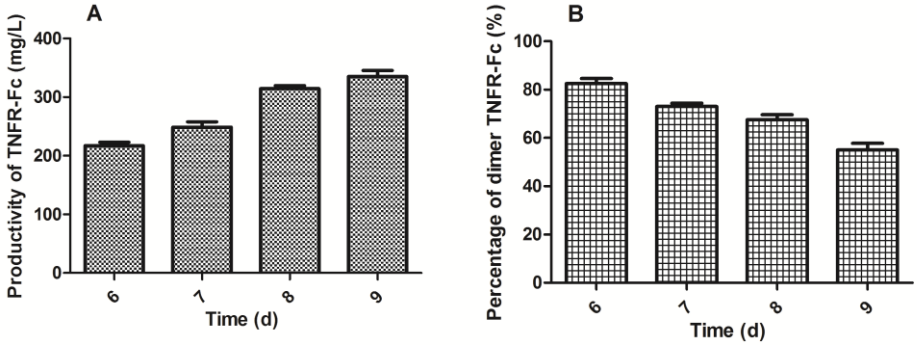

Fig. 2 Effect of culture time on TNFR-Fc productivity and qulity

3.2 Effect of Culture Time on TNFR-Fc Productivity and Qulity

As is shown in figure 2, concentration of TNFR-Fc was more than 200 mg/L on the 6th day and reached to 330 mg/L three days later. However, the percengtage of TNFR-Fc dimer decreased from 82% to 55% during this time which meant the quality of TNFR-Fc became worse.

A.TNFR-Fc productivity. B.Dimeric TNFR-Fc percentage. Productivity of TNFR-Fc was measured by ELISA and percentage of TNFR-Fc was calculated by SEC-HPLC.

3.3 Analysis of purified TNFR-Fc

After purifying culture medium with protein A column, we used SEC-HPLC to collect purification fraction respectively. The retention time of first peak was at 12.02 min which means molecular weight is larger than dimeric TNFR-Fc, while dimer was at 14.00 min followed by smaller molecule. Fraction collection was performed by four parts as is shown in figure 3.

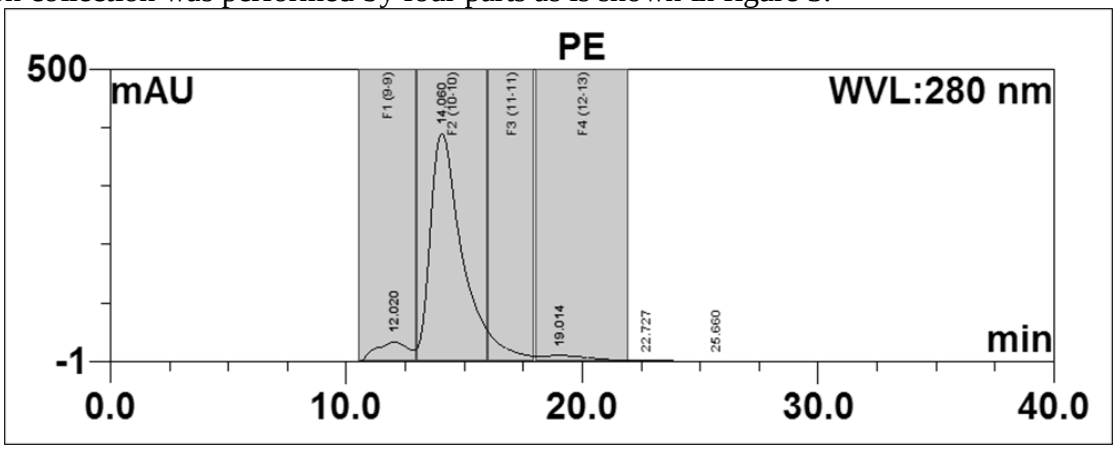

Fig. 3 Fraction collection after purification

Culture medium was purified by protein A column, and SEC-HPLC was used to collect purification fraction respectively. The peak was divided into four parts as marked in the figure.

Non-reduction SDS-PAGE was performed to detect the molecular weight of the four fractions. The first fraction main included polymeric TNFR-Fc conjugated with covalent bond. And the polymeric TNFR-Fc was a kind of misfolding protein and might have an effect on the quality of TNFR-Fc. The second fraction was dimer which was the desirable component. And the content is the most among the four fractions. The 3rd and 4th fractions were smaller molecules which indicated they were the digestion of dimeric TNFR-Fc.

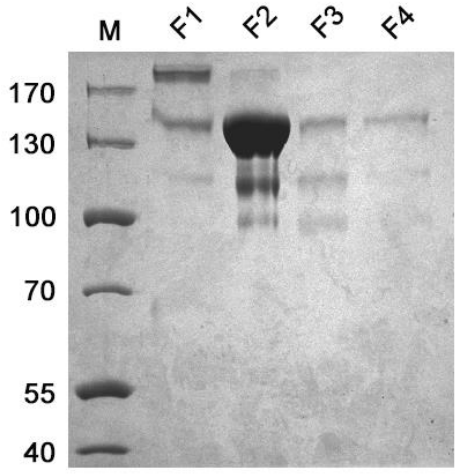

Fig. 4 Fraction Detection

Fractions were detected by SDS-PAGE after SEC-HPLC collection.

4. Discussion

Dimeric TNFR-Fc aggregates further leading to the formation of polymer. TNFR-Fc aggregation is a main reason for its impurity during producing process. TNFR-Fc aggregation has more Fc fragments and binds to protein A column tightly. That is why TNFR-Fc aggregation cannot be excluded effectively by affinity column chromatography. So other purification methods have to be adapted, such as ion exchange column chromatography and hydrophobic chromatography, which will take more time and money to get desirable product. If TNFR-Fc aggregation is controlled during producing process, some complicated purification process can be saved.

5. Conclusion

During the later period of CHO cell culture, CHO cell viability went down which meant cell living condition becoming unsuitable. Although the productivity of TNFR-Fc continued to increase, the percentage of dimeric TNFR-Fc decreased at the same time. After protein A column affinity purification, the sample contain three parts, namely polymeric and dimeric TNFR-Fc as well as TNFR-Fc digestion. If cell living condition becomes worse, there will be less dimeric TNFR-Fc, however, more polymer and digestion.

Acknowledgements

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