Creating a New Medium to Help Meet the Variable Nutritional Requirements of Chinese Hamster Ovary (CHO) Cell Clones

Z.W. Deeds, A. Albee, B. Delong, J. Gifford, J.S. Ross, K. Kao, and M.V. Caple
Sigma-Aldrich Biotechnology, 3050 Spruce Street, Saint Louis, MO 63103 USA

Abstract

It is widely known that different recombinant protein expressing Chinese Hamster Ovary (CHO) cell clones can have variable nutritional requirements. Sigma-Aldrich has previously developed an Animal-Component Free Medium (Product Code C5467) that for many CHO clones yields excellent growth and productivity. However, with some CHO clones it yields less than optimal performance. Therefore the need has arisen to create a second-generation medium that will complement C5467.

The Dihydrofolate Reductase (DHFR) gene amplification system has become very popular for use in CHO clones. The DHFR system allows the gene of interest to be amplified with increasing concentrations of methotrexate, leading to increased protein expression. The recombinant cell lines used in the optimization of this new medium were all DHFR clones expressing a variety of protein products, including a rIgG and rhM-CSF. Each cell line poses a unique challenge, as demonstrated by the differences in amino acid utilization rates.

Based on cell growth, r-protein production, and the amino acid consumption rates, CHO DHFR- Medium (Product Code C8862), was developed. The base formulation for C8862 was very different from that of C5467, allowing for the development of a complementary animal-component free medium. Experiments have shown that C8862 promotes better growth and protein production than C5467 with many CHO clones, but there are still other cases where using C5467 is advantageous. The development of this new medium resulted in growth and recombinant protein production rates surpassing that of other commercially available media. Finally, the application of these two CHO animal-component free media in spinner flasks and 5-L bioreactors is shown.

Introduction

Chinese Hamster Ovary (CHO) cells are one of the most frequently used cell lines for the expression of recombinant proteins that require post-translational modification to express full biological function. Since more and more biopharmaceutical companies are producing their potential therapeutic agents in CHO cells, there has been increased regulatory scrutiny of the medium in which the cells are grown. Animal component-free media have now come to the forefront for use with CHO cells. Sigma has previously developed a CHO Animal Component-free Medium (Product Code C5467), to support the suspension culture of CHO cells and to achieve the desired recombinant protein expression. This CHO medium contains recombinant human insulin, plant hydrolysates, and proprietary iron chelators. All other components are also of non-animal origin, including amino acids, vitamins, fatty acids and surfactants. C5467 has proven to be medium of choice for many recombinant protein producing cell lines, but not for all.

In order to obtain maximal growth and recombinant protein production with those clones for which C5467 is not optimal, Sigma has created CHO DHFR-Medium (Product Code C8862). Much like C5467, CHO DHFR-Medium contains recombinant human insulin, plant hydrolysates, and proprietary iron chelators and is completely animal-component free. Many factors were considered while CHO DHFR- Medium was being developed, including amino acid analysis. This information allowed insight into the utilization rates of different cell lines.

The recombinant cell lines used in the development of this medium were all DHFR (Dihydrofolate reductase) clones. This cloning system allows one to greatly increase the gene of interest copy number within their specific clone, leading to increased recombinant protein expression. The cell lines that were studied produced either a recombinant antibody or a recombinant human growth factor.

Finally, CHO DHFR- Medium is compared to other commercially available media and is also tested in bioreactor experiments.

Materials and Methods

Sigma-Aldrich Corporation (St. Louis, MO) supplied all chemicals, media and solutions unless otherwise stated.

Cell Lines

CHO 5/9 m alpha 3-18 (M-CSF) cells (ATCC #CRL-10154) were obtained from the American Type Culture Collection (ATCC). Cell line CHO-IgG is a proprietary clone expressing a recombinant antibody.

Culture media

The media used in this study are CHO Animal Component-Free Medium (C5467) and CHO DHFR- Medium (C8862).

Cell Culture and Cell Growth Assays

Cells were grown in suspension in C5467 and were used to seed experiments done in 125ml or 250ml (100ml and 150ml liquid volume respectively) Techne Spinner flasks. The initial inoculum was 50,000 viable cells per milliliter. All conditions were run in duplicate. The cells were cultured in Forma incubators at 37 °C and 5% CO_2.

For bioreactor experiments, B. Braun Biotech International's Biostat® B five liter reactors were used. They were set-up with marine impellers and a 0.5 µm sparging stones. These were also seeded at 50,000 viable cells/mL.

Spent medium samples were collected every day for the analysis of nutrients/metabolites and recombinant protein production. At the same time, the cells were counted using a Schärfe System Casy 1® Model TTC and viability was assessed using the Trypan Blue Exclusion Method.

Quantification of Recombinant Humanized IgG and Recombinant Human M-CSF

The IgG secreted into the medium by CHO IgG was measured by HPLC (Waters 2690 HPLC Millipore, MA) using a protein-G affinity column. The analysis is an affinity chromatography method, utilizing an analytical column packed with poly-flow through particles designed for very rapid mass transport. The protein-G has a high affinity for IgG under neutral conditions. The column does not retain other proteins such as albumin. The bound IgG is then quickly removed with an acidic solution. The amount of IgG in the subsequent peak is detected and quantified by UV absorbance at 210 nm.

Recombinant Human M-CSF was measured by a Quantikine® Human M-CSF Immunoassay supplied by R&D Systems (Catalog # DMC00).

Results

Growth and recombinant IgG Production with CHO-IgG clone

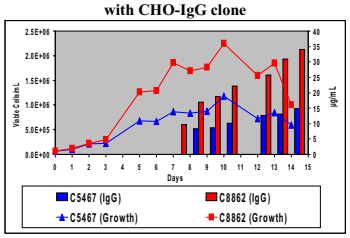

Figure 1: Comparison between C5467 and C8862 for growth and recombinant IgG production. In this experiment CHO-IgG cells, grown in C5467, were seeded in Techne spinner flasks (250mL). The results show that C8862 supports much better cell growth and recombinant IgG production.

Growth and recombinant hM-CSF Production with CHO-M-CSF clone

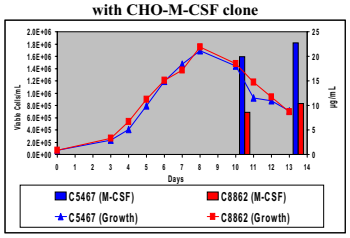

Figure 2: Comparison between C5467 and C8862 for growth and recombinant M-CSF production. In this experiment CHO-M-CSF cells, grown in C5467, were seeded in Techne spinner flasks (125mL). The results show that the growth in C5467 and C8862 were very similar, with a much enhanced production of rh-M-CSF in C5467.

Amino Acid Analysis - CHO-M-CSF in C8862

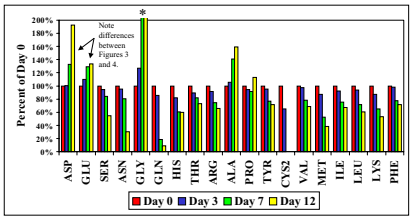

Figure 3: Amino Acid Analysis. Spent medium samples were collect from an experiment preformed in spinner flasks that used the CHO-M-CSF line in C8862 and then analyzed for amino acid content. The data is presented here as a percent of Day 0 values. Many differences between Figures 3-5 can be noted, demonstrating the variable nutritional requirements of CHO cell clones and the different utilization rates in different media.

*Glycine Day 7 = 279%, Day 12 = 622%

Amino Acid Analysis - CHO-IgG in C8862

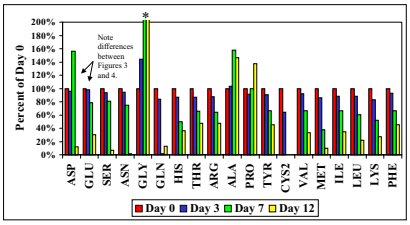

Figure 4: Amino Acid Analysis. Spent medium samples were collect from an experiment preformed in spinner flasks that used the CHO-IgG line in C8862 and then analyzed for amino acid content. The data is presented here as a percent of Day 0 values. Many differences between Figures 3-5 can be noted, demonstrating the variable nutritional requirements of CHO cell clones and the different utilization rates in different media.

*Glycine Day 7 = 420%, Day 12 = 846%

Amino Acid Analysis - CHO-IgG in C5467

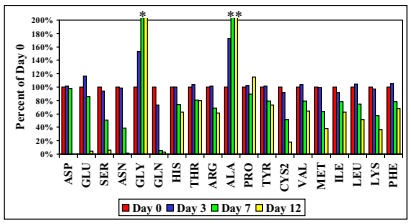

Figure 5: Amino Acid Analysis. Spent medium samples were collect from an experiment preformed in spinner flasks that used the CHO-IgG line in C5467 and then analyzed for amino acid content. The data is presented here as a percent of Day 0 values. Many differences between Figures 3-5 can be noted, demonstrating the variable nutritional requirements of CHO cell clones and the different utilization rates in different media.

*Glycine Day 7 = 367%, Day 12 = 776% **Alanine Day 7 = 630%, Day 12 = 833%

Media Comparison - CHO-IgG

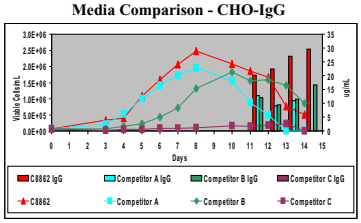

Figure 6: Performance Comparison with CHO-IgG clone. Growth and recombinant IgG production were analyzed in this spinner experiment (125mL) that compares C8862 to other commercially available media. C8862 yielded the highest for both.

Media Comparison - CHO-M-CSF

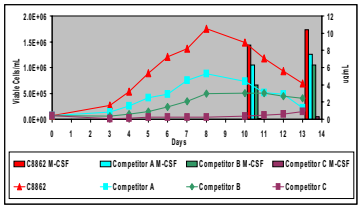

Figure 7: Performance Comparison with CHO-M-CSF clone. Growth and recombinant human M-CSF production were analyzed in this spinner experiment (125mL) that compares C8862 to other commercially available media. Again, C8862 yielded the highest for both.

Media Comparison – Specific Productivity

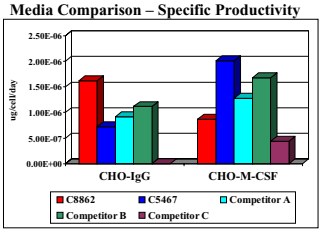

Figure 8: Specific Productivity. This data is taken from Day 13 of a spinner experiment (125mL) using both the CHO-IgG and CHO-M-CSF cell lines.

Bioreactors with CHO-IgG in C8862

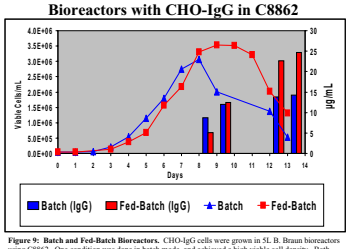

Figure 9: Batch and Fed-Batch Bioreactors. CHO-IgG cells were grown in 5L B. Braun bioreactors using C8862. One condition was done in batch mode, and achieved a high viable cell density. Both growth and r-IgG production were enhanced using a fed-batch mode, where glucose and glutamine were added to the culture and kept above 3 g/L and 2mM respectively.

Discussion

Sigma's new product, CHO DHFR- Medium (C8862), was developed in response to data suggesting that our CHO PF AF Medium (C5467) was not performing as expected with a significant amount of recombinant CHO cell clones. This is demonstrated in Figure 1, where C5467 yields sub-optimal growth and productivity with the CHO-IgG cell line. C8862 is a vast improvement over this medium when considering the CHO-IgG clone. However, the variable requirements of different CHO clones are clearly shown by the results in Figure 2. Here the CHO-M-CSF cell line grew similarly in both media, but the rh-M-CSF production was much better in C5467.

Furthermore, amino acid analysis gives even deeper insight into the variations amongst CHO clones. Aspartic acid (ASP) utilization is one example. Comparing the CHO-IgG and CHO-M-CSF cell lines in C8862 (Figures 3 and 4), one sees that the aspartic acid level in both cultures has increased by day 7. This continues with the CHO-M-CSF cell line, to almost double the initial amount by day 12. However, the trend reverses with the CHO-IgG cell line, and by day 12 almost all of the available aspartic acid is utilized. Another interesting note is that with the CHO-IgG cell line, differences can be seen in the amino acid utilization rates with the different media (Figures 4 and 5). Again using aspartic acid as an example, we can see that in C5467 the increase in concentration is not seen on day 7, but much like C8862, most of the available aspartic acid is gone by day 12.

Figures 6 and 7 show how CHO DHFR- Medium compares to other commercially available media. With both cell lines, C8862 yields the highest growth and total recombinant protein production.

Specific productivity (Figure 8) further elucidates the need for multiple medium formulations. With the CHO-IgG cell line, C8862 had the highest specific productivity while C5467 was more in the range of the competitors. Conversely, with the CHO-M-CSF cell line C5467 yielded the highest specific productivity.

Scalability of C8862 was demonstrated in 5L B. Braun bioreactors, in both batch and fed-batch modes using the CHO-IgG cell line (Figure 9).

Conclusions

- Given the variable nutritional requirements of recombinant CHO cell clones, one medium can not supply the necessary nutrients to support maximal growth and recombinant protein production with all clones.
- CHO DHFR- Medium (C8862) has been shown to increase growth and recombinant protein production with CHO cell lines that did not perform well with Sigma's CHO Protein-Free Animal Component-Free Medium (C5467). However, there are still cases where C5467 is optimal.
- Amino acid utilization rates can yield deeper insight into the variability amongst CHO cell clones.
- Sigma's C5467 and C8862 media possess significantly diverse characteristics as indicated by the different amino acid utilization patterns with the same CHO clone.
- According to the data presented, CHO DHFR- Medium (C8862) performs well when compared to other commercially available media for growth and recombinant protein production with CHO clones.
- Scalability with CHO DHFR- Medium has been demonstrated in 5-liter bioreactors.

Acknowledgements

We would like to thank all who contributed to the research and development of CHO media at the Sigma-Aldrich Saint Louis R&D facility.

SIGMA-ALDRICH