The Effects of Media Formulations on the Biochemical Profile of IgG Expressed in Sp2/0 Cells as Measured by Cation Exchange HPLC

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Cell Culture
Sp2/0 cells were grown in suspension cultures in shake flasks at 37°C, 100 rpm, using a shaking incubator at a speed of 150 rpm. Cells were seeded at an initial density of 1×10^8 cells/ml with medium containing 10% fetal bovine serum. For growth control, media were added to each plate daily to a final volume of 200 μL. For fed-batch studies, media were initially added to each plate daily to measure viable cell density and at the end of the study to evaluate product purity. Media formulations were used to control the pH of the medium, which was monitored daily and adjusted as needed with sodium carbonate.

Methods

Media Selection

IgG was concentrated and purified using Protein A resin (G.E. Healthcare, UK) in a 96 well filter plate. The pH was monitored daily and adjusted as needed with sodium carbonate. The pH was maintained and adjusted as needed with sodium carbonate.

IgG Sample Preparation and Stoichiometry Determination

Sample concentration was measured using a 280-nm Absorbance UV-Vis spectrophotometer. The absorbance of the sample was determined to be 0.100 at 280 nm. The sample was then diluted with water to a final concentration of 1 mg/mL for analysis.

Figure 3: Normalized media screening also demonstrated that growth with different formulations had significant effects on the percentage of acidic fractions. The acidic fraction was defined as the percentage of the total area under the curve (TACU) that was below 80% of the total TACU.

Figure 4: A sample of MAb H48C containing a molar ratio of acidic fraction % acidic (5:4) was treated with 90 μL of sialidase (50,000 U/mL; New England Biolabs, MA, USA) and incubated for 24 h at pH 7.0. Sialidase treatment resulted in a reduction of the % acidic fraction from 4.1% to 2.6%.

Figure 5: Cells were seeded into shake flasks containing different media formulations described in Table 2 and cultured for 10 days. Media formulations represented by different colors in the figure were spectrophotometrically determined. The medium was removed from the flasks and replaced with new media containing the desired CEX HPLC profile. This approach was followed for each media formulation in the experiment.

Figure 6: CEX HPLC chromatograms from media formulations used in a previous study showed that the % acidic fraction varied from 1.2% to 4.0%.

Figure 7: The effects of media formulations on the biochemical profile of IgG were measured by Cation Exchange HPLC. The samples were tested for the presence of acidic and basic fractions.

Table 3: Summary of data from media screening experiments. The biochemically defined CEX fractions were identified and the percentage of acidic and basic fractions determined. The data was then used to calculate the % acidic fraction.

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Summary

Our studies have demonstrated that changes in media formulations can have dramatic effects on the charge heterogeneity of a recombinant MAb expressed in Sp2/0 cells. Using a CEX HPLC assay, we detected significant changes in the ratios of the OK, 1K, and 2K peaks when the MAb was produced in different media formulations. In addition, the amount of IgA leaking into the acidic fraction could vary dramatically. Changes in the acidic fraction seemed to follow several potential mechanisms, including changes in the overall pl of the MAb, changes in the expression levels of MAb isoforms, and changes in the production of sialic acid. The results suggest that media optimization is necessary to achieve a more stable production process and to ensure the quality of the final product.

References