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Optimization of chemically defined feed media for monoclonal antibody production in Chinese hamster ovary cells

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Chinese hamster ovary (CHO) cells are the most commonly used mammalian host for large-scale commercial production of therapeutic monoclonal antibodies (mAbs). Chemically defined media are currently used for CHO cell–based mAb production. An adequate supply of nutrients, especially specific amino acids, is required for cell growth and mAb production, and chemically defined fed-batch processes that support rapid cell growth, high cell density, and high levels of mAb production is still challenging. Many studies have highlighted the benefits of various media designs, supplements, and feed addition strategies in cell cultures. In the present study, we used a strategy involving optimization of a chemically defined feed medium to improve mAb production. Amino acids that were consumed in substantial amounts during a control culture were added to the feed medium as supplements. Supplementation was controlled to minimize accumulation of waste products such as lactate and ammonia. In addition, we evaluated supplementation with tyrosine, which has poor solubility, in the form of a dipeptide or tripeptide to improve its solubility. Supplementation with serine, cysteine, and tyrosine enhanced mAb production, cell viability, and metabolic profiles. A cysteine–tyrosine–serine tripeptide showed high solubility and produced beneficial effects similar to those observed with the free amino acids and with a dipeptide in improving mAb titers and metabolic profiles.

[**Key words:** Amino acid; Chemically defined medium; Chinese hamster ovary cells; Monoclonal antibody; Tripeptide]

Chinese hamster ovary (CHO) cells are commonly used mammalian hosts for the commercial production of therapeutic proteins such as monoclonal antibodies (mAbs) (1), which are commercially the fastest growing types of new therapeutic molecules, owing to their high antigenic specificity and low incidence of undesirable side effects. Although mAbs have proven to be therapeutically useful, the typical doses required for treatment are substantially higher than those required for most other biologics. Therefore, mAbs must be produced on a large scale via efficient, cost-effective processes so that the expense to patients can be reduced. Recently, mAb production has been enhanced by improvement and refinement of clone selection, expression vectors, transfection technologies, and cell culture media. In optimized fed-batch processes, expression levels of 1–5 g/L mAb are now typically achieved (2–4). However, further process enhancement is still necessary to reduce production costs and expense to patients.

Cell culture media (which typically consist of amino acids, vitamins, trace metals, sugars, salts, buffers, growth factors, and various other components) have been shown to affect cell growth (5), antibody titer (6), and product quality (7). Methods for the optimization of cell culture media, such as the use of nutritional supplements and additives, are important for improving metabolism, cell survival and productivity, and protein expression levels.

CHO cells are typically cultivated in growth media supplemented with 1%–20% fetal bovine serum, which is rich in the nutrients and growth factors necessary for cell growth and productivity (8). However, the use of serum raises significant regulatory concerns owing to the risk of contamination by animal-derived materials such as viruses (9). To minimize this risk, hydrolysates are widely used as serum replacements in the production of therapeutic proteins. Although hydrolysates (peptones of soy, rice, wheat, cotton and so on) can improve cell growth and protein yield in serum-free processes (10–13), they have some disadvantages, such as lot-to-lot variation (14,15) because they are complex, undefined raw materials derived from plant or other non-animal sources. Therefore, current approaches in medium development focus on chemically defined media for the production of recombinant proteins and mAbs in CHO cells. Several chemically

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defined media for NS0 cell and CHO cell cultures have been developed (16–19), but chemically defined media that work in fed-batch processes and that support rapid cell growth, high cell density, and the production of high levels of recombinant proteins and mAbs are still unsatisfactory.

In the present study, we developed a strategy for optimizing a chemically defined feed medium based on spent media analysis of amino acids to improve mAb titers (20). Spent media analysis provides important information on how a medium changes during the culture process. By comparing spent medium samples with a fresh sample, we quantitatively evaluated both nutrient depletion and metabolite accumulation and then selected the various amino acids that were consumed during the control culture experiment. The reason for examining amino acids here was that they are the main constituents of chemically defined media and are easy to manipulate (21).

MATERIALS AND METHODS

Cell line and cell culture conditions We used DUK-XB11 (DXB11, a gift of Dr. L. Chasin) CHO cells that had been adapted in CHO-S-SFM II medium (Life Technologies, Grand Island, NY, USA). The expression system was based on dihydrofolate reductase amplification (22,23). The cells were transfected with a plasmid encoding IgG mAb and were passaged regularly in mixed CHO-S-SFM II–CD-CHO medium (50:50) (Life Technologies) supplemented with 50 nM methotrexate. Cells were subcultured in suspension in 100-mL spinner flasks (Bellco Glass, Vineland, NJ, USA) in a CO_2 incubator (Thermo Scientific, Waltham, Massachusetts, USA). The cultures in 100-mL spinner flasks were kept at 37°C in a humidified atmosphere with 5% CO_2. All production cultures were performed in fed-batch mode with continuous feeding; model BCP bioreactors (125 mL; Able, Tokyo, Japan) were used for production cultures. Cells were inoculated at 2.0×10^5 cells/mL into the bioreactors. The dissolved oxygen concentration was controlled at 40% of air saturation, and the pH was controlled at 7.2 until the end of day 2, and then at 6.9 thereafter, with a mixed 1 M NaOH–1 M $NaHCO_3$ (50:50) solution and CO_2. Continuous feeding was started on day 3.

Basal medium, feed medium, and peptides CHO-S-SFMII–CD-CHO medium was used for passage and production culture. Three-fold concentrated CHO-S-SFMII–CD-CHO medium was used as the feed medium. Chemically synthesized dipeptide and tripeptide were purchased from Life Technologies.

Analytical methods Cell count and cell viability determinations were performed using a CEDEX model AS20 (Roche Innovatis, Bielefeld, Germany) automated cell counting device using trypan blue exclusion. Amino acid and ammonium concentrations were measured by means of high-performance liquid chromatography (HPLC) using ninhydrin reagent and an improved Hitachi L-8500 amino acid analyzer (Hitachi, Tokyo, Japan) (24). Glucose and lactate concentrations were assayed with a biochemistry analyzer model YSI 2300 (YSI Life Sciences, Yellow Springs, OH, USA). Protein-A HPLC with appropriate reference standards was used to measure mAb titers. All analytical methods were validated and the coefficients of variation were less than 5%.

RESULTS AND DISCUSSION

Effects of amino acid supplementation on mAb titer, viability, and lactate and ammonium concentrations To determine the effects of amino acid supplementation on mAb titer, we tested five amino acids (asparagine, glutamine, serine, cysteine, and tyrosine) by using a chemically defined medium and a fed-batch process. These five amino acids were selected because their concentrations decreased markedly during a control culture experiment (Fig. 1). All five amino acids were used in the group 1 experiments. In contrast, in the group 2 experiments, only three amino acids (serine, cysteine, and tyrosine) were added to the feed medium as supplements; asparagine and glutamine were omitted from the group 2 experiments to avoid ammonium accumulation due to catabolism of the amine-containing side chains of these two amino acids. The concentrations of asparagine, glutamine, serine, cysteine, and tyrosine added to the feed medium were calculated on the basis of the spent media analysis and were 35.0, 84.0, 50.0, 1.8, and 14.5 mM, respectively. These concentrations were sufficient to maintain adequate amino acid levels during the culture (data not shown).

We first elucidated profiles for viable cell concentration and viability (Fig. 2A). In the control culture, the maximum viable cell concentration was 51×10^5 cells/mL on day 5, whereas those in group 1 and 2 on day 5 were only 43×10^5 cells/mL and 45×10^5 cells/mL, respectively. Viability in group 1 dropped sharply after day 10 and was lower than that of the control group at day 12. In contrast, viability in group 2 was higher than that in the control group. The culture-time dependences of the mAb titers in the two experimental groups and the control group were similar until day 7; after that time, the titer started to level off in group 1, whereas the levels in the control group and group 2 continued to increase linearly until the end of the culture period. By day 12, the mAb titer in group 1 was 30% lower than that in the control group (Fig. 2B). In contrast, the mAb titer in group 2 was 20% higher than that in the control group. The differences in the mAb titers can primarily be attributed to changes in relative specific mAb productivity (qmAb). In group 1, relative qmAb was 0.54, which was 46% less that in the control group. In contrast, in group 2, relative qmAb was 1.39, which was 39% more than that in the control group (Fig. 2C). The lactate concentration in group 2 stayed below 1.5 g/L throughout the culture period (Fig. 2D). The ammonium concentration in group 1 reached 540 mg/L by the end of the culture period, owing to catabolism of asparagine and glutamine (Fig. 2E). On day 12, the osmolalities of group 1 and 2 were approximately 250 mOsm/kg and 105 mOsm/kg higher, respectively, compared with that of the control group (Fig. 2F).

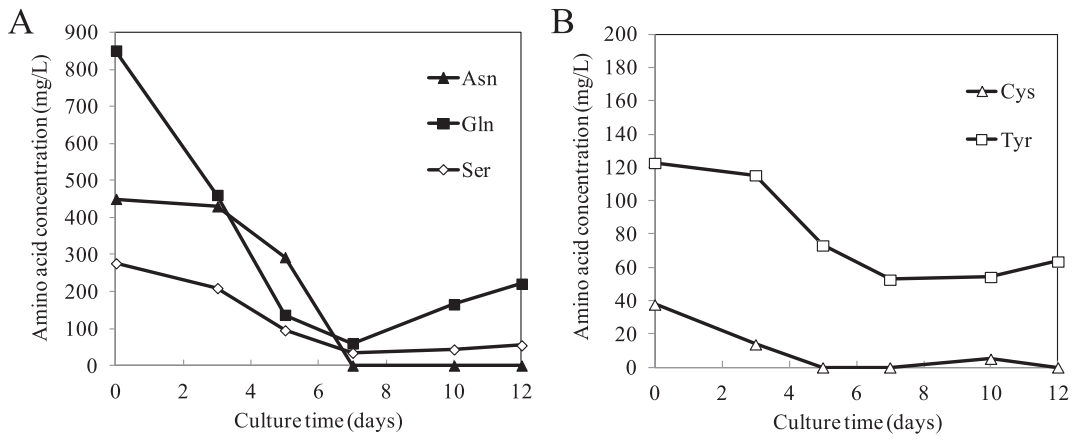

FIG. 1. Culture-time dependence of amino acid concentrations in the control culture: (A) asparagine (Asn), glutamine (Gln), and serine (Ser) and (B) cysteine (Cys) and tyrosine (Tyr).