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Development of serum-free media for a recombinant CHO cell line producing recombinant antibody

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Abstract

A serum-free medium (SFM-MDM) without any animal-driven protein was developed for the production of recombinant antibody in suspension cultures of recombinant Chinese hamster ovary cell line, CS*13-1.00. The formulation of SFM-MDM was based upon the Iscove's modified Dulbecco's medium (IMDM), and Long^{TM}R^{3}IGF-1 and ferric citrate were supplemented instead of insulin and transferrin. Among various protein hydrolysates, yeast extract was selected as a serum-substituting additive for the improvement cell growth and productivity of recombinant antibody. Through about 3600 h of adaptation period from conventional SFM to SFM-MDM, integral of viable cell density and specific antibody productivity increased from 7.60×10^6 cells d and 19.14 μg $(10^6$ cells d$)^{-1}$ to 7.79×10^6 cells d and 54.04 μg $(10^6$ cells d$)^{-1}$, respectively, resulted in final antibody concentration of 420.98 mg l^{-1}, which was about three times higher than that obtained from commercial medium, 486F. Also, supplementation of various vitamins including biotin, pyridoxal·HCl, and ascorbic acid was observed to enhance specific productivity, which led to high final antibody concentration. These results suggested the fundamental and effective strategy for development of a serum-free medium and to enhance cell growth and production of target proteins.

Keywords: Serum-free medium; Recombinant antibody production; Chinese hamster ovary cell

1. Introduction

There have been lots of efforts to develop serum-free/protein-free media for the production of therapeutic proteins by mammalian cells [1–5]. Serum contains various proteins, vitamins, hormones, growth factors, lipids, and other trace elements, which support the growth of mammalian cells [1]. Therefore, determination of serum-substituting components has been one of the most significant considerations in serum-free/protein-free media developments.

Serum-free/protein-free media can protect contamination by bacteria, virus, and mycoplasma which can be included in a serum-containing medium and make a cost-effective operation possible in a large scale process [1]. Also, it is encouraging to utilize serum-free/protein-free medium in suspension cultivation of mammalian cells for the rational process development. Since quality assurance can be accessed with serum-free medium, its accurate formulation plays an essential role in downstream processing and reproducibility of target products. Also, defined formulation of serum-free medium provides fundamentals to develop a production medium for fed-batch or perfusion cultures [2–5].

Previous research groups introduced several methods for development of serum-free media. To enhance cell growth and production of target proteins, basal formulation was improved [1–4] and various systematic approaches were evaluated for synergic effects of many serum-substituting components [5,6]. Those methods showed that depletion or excess of even single

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component in the medium could induce the cell apoptosis followed by low productivity. Balanced supplementation of limiting components including vitamins, amino acids, and hormones was considered as a very important factor in development of serum-free/protein-free medium [7].

In this study, a new serum-free medium for the cultivation of recombinant Chinese hamster ovary cell, CS*13-1.00 producing recombinant antibody was designed with a simple but efficient strategy. To avoid usage of animal-derived proteins and to prevent cell aggregation during culture period, various medium components were appraised and most suitable concentration of each component was proposed for enhancement of the cell growth and the productivity of recombinant antibody.

2. Materials and methods

2.1. Cell lines and media

A recombinant CHO cell line, CS*13-1.00, expressing a chimeric antibody against the S surface antigen of Hepatitis B virus was used. It was established by co-transfection of plasmids expressing light and heavy chain into dihydrofolate reductase (DHFR)-deficient CHO cells (DG44-origin), followed by subsequent DHFR/methotrexate (MTX)-mediated gene amplification, and was kindly donated by Animal Cell Engineering Laboratory in KAIST. The working clone of rCHO CS*13-1.00 was selected at 1.00 μM MTX. Suspension adaptation of rCHO cell was performed in a conventional protein-free medium 486F (WelGEN, Daegu, Korea).

2.2. Culture conditions

Batch suspension culture was established in a 150 ml Erlenmeyer flask (Corning Corp., Corning, NY) with an inoculum of 2.0×10^5 cells ml^{-1} in working volume of 50 ml. A gyratory shaker (n-Biotech, Incheon, Korea) at 110 rpm was used. The culture was maintained at 37 °C in 5% CO_2 atmosphere.

2.3. Medium additives for nutrient fortification

Most of amino acids (Ajinomoto Co., Inc., Tokyo, Japan) and water soluble vitamins (Sigma, St. Louis, MO) except for folic acid and riboflavin were dissolved at approximately 100-fold basal medium levels in deionized water. Folic acid (Sigma) and Riboflavin (Sigma) were dissolved in basal medium. Long^{TM}R^{3}IGF-1 (GroPep, Adelaide, Australia) was diluted in deionized water and hydrocortisone, phospatidylcholine, and all liposoluble vitamins (Sigma) were dissolved in pure ethanol. Other chemicals used were of reagent grade and were obtained from either Junsei Chemical Co. (Tokyo, Japan) or Sigma Chemical Co. and were dissolved in deionized water. All components were stored at −20 °C until used.

Soy protein hydrolysate (HyPep 1510), wheat protein hydrolysate (Hypep 4602), yeast extract (Hypep 7455), and mixture of hydrolysate (SR3) were magnanimously provided by Quest international (Hoffman Estate, IL) and animal component-free protein hydrolysate was purchased from WelGEN (Daegu, Korea).

2.4. Analytical methods

Viable and dead cells were directly counted with a hemacytometer, using the dye exclusion method (0.4% Trypan blue in phosphate buffered saline). Glucose and lactate concentration were measured with YSI 2700 SELECT Biochemistry Analyzer (YSI Inc., Yellow Springs, OH).

Recombinant antibody concentration was assayed by System Gold HPLC equipped with 166 UV detector (Beckman Instrument, San Ramon, CA) and a POROS A affinity column (Applied Biosystems, Foster City, CA). A pH gradient from two buffers (pH 7.3, pH 1.3) was adopted to isolate the peak of immunoglobulin.

Amino acids were quantified by system Gold HPLC equipped with 166 UV detedtor (Beckman Instrument) and C-18 column (Waters, Milford, MA) using FMOC derivatives. Buffer solution with 50 mM sodium acetate (pH 4.2, 80%) and acetonitrile (20%) and buffer solution with 50 mM sodium acetate (pH 4.2, 20%) and acetonirile (80%) were also adopted to isolate the peaks of amino acids.

The osmolality and pH of the medium supplemented with various additives were measured with the Advanced^{TM} Micro Osmometer Model 3300 (Advanced Instrument, Inc., Nonwood, MA) and pH meter MP220 (Mettler Toledo, Columbus, OH), respectively.

3. Results and discussion

3.1. Development of basal serum-free medium

Basal serum-free medium (SFM) was designed for a recombinant CHO cell line, CS*13-1.00, producing recombinant anti-

Table 1
Determination of basal medium component

Exp. group	IMDM supplemented with	mg l^{-1}	Cell growth
A	$Fe(NO_3)_3 \cdot 9H_2O$	2	–
	$CuCl_2$	0.0025	
	$ZnSO_4 \cdot 7H_2O$	1	
	Pluronic F-68	1000	
B	A + serum	–	+++
C	A + Long^{TM}R^{3}IGF-1	0.02	+
	Na_2SeO_3	0.0173	
	Ferric citrate	2	
	Ethanolamine	3	
	Phospatidylcholine	5	
D	C + soy protein hydrolysate (HyPep 1510)	5000	++
E	C + wheat protein hydrolysate (HyPep 4602)	5000	–
F	C + yeast extract (HyPep 7455)	5000	+++
G	C + mixture of hydrolysate (SR3)	5000	–
H	C + animal component-free protein hydrolysate	5000	–
I	Commercial medium (486F)	–	+++

(+) Degree of cell growth ('++' means 10% higher than '+' in cell density).