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Effects of supplementation of various medium components on Chinese hamster ovary cell cultures producing recombinant antibody

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Abstract

Thirteen vitamins, twenty amino acids, hormones, inorganic salts, and other chemical agents, which constitute typical serum-free media, were evaluated for the development of fortified medium to enhance cell growth and productivity of recombinant antibody in the cultures of the recombinant Chinese hamster ovary (rCHO) cells. Two different rCHO cell lines, rCHO-A producing recombinant antibodies against the human platelet and rCHO-B secreting recombinant antibodies against the S surface antigen of Hepatitis B, respectively, were cultivated in batch suspension mode. Concentration of interested component in the tested medium was doubled to examine the fortification effect. Growth of rCHO-A cell and its antibody production were slightly improved with addition of either choline chloride, folic acid, thiamine·HCl, or Long^{TM}R^{3}IGF-I. On the other hand, in the cultivation of rCHO-B cell which was more sensitive to its environmental changes, hormones such as Long^{TM}R^{3}IGF-I and triiodothyronine (T_3) as well as various vitamins involving choline chloride, i-inositol, niacinamide, pyridoxine·HCl, and thiamine·HCl enhanced the cell growth and antibody production. Particularly, when concentration of consuming amino acid was doubled, remarkable increase in specific productivity was served, resulting in high final antibody concentration. These results were believed to provide a fundamental strategy of medium fortification useful for improvement of recombinant antibody production in serum-free medium.

Introduction

Therapeutic proteins derived from mammalian cells such as recombinant antibodies have been used not only as disease treating therapeutics but also as diagnostics (Oh et al. 1994) and *in vivo* imaging agents (Bibila et al. 1995). Recombinant antibodies received a great deal of attention as a prominent product through biotech pipelines toward the marketplace. They now occupy about 25% of the estimated medicines in clinical development and many more antibodies which lead the value of the market going forward are reported (Lee et al. 2005).

There has been much effort to identify suitable serum-substituting components due to the

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increasing needs for serum-free and protein-free media (Lee et al. 1999; Chen et al. 2000; Schröder et al. 2004; Sung et al. 2004; Lee et al. 2005). Therefore, to determine the composition of culture medium is one of the most important considerations in serum-free cultivation of mammalian cells. Animal cells require various nutrients such as amino acids, vitamins, hormones, inorganic salts, and diverse undefined components to survive *in vitro* and produce the target therapeutic proteins efficiently. Because most of mammalian cells are very sensitive to their environmental change, balanced addition of limiting components with a detailed medium analysis take a critical portion in the modification of medium formulation (Stoll et al. 1996; Ducommun et al. 2001). Additional supplementation of amino acids and vitamins to serum-free or protein-free media has resulted in remarkable increases in viable cell density and final recombinant antibody production (Stoll et al. 1996; Ducommun et al. 2001; Takagi et al. 2001; Ishaque and Al-Rubeai 2002). Also, various hormones, inorganic salts, and other chemical reagents are known to improve the cell growth and productivity of therapeutic proteins when they are added to the media (Morris and Schmid 2000; Sunstrom et al. 2000). Also, several strategies for the fortification of basal media have been developed to improve culture longevity (Stoll et al. 1996; Ducommun et al. 2001).

Since universal serum-free/protein-free medium suitable for all cell lines is not existed (Kim 1998) and effective medium components are different for each cell line, it is important to appraise general components which constitute serum-free/protein-free at a time in the recombinant Chinese hamster ovary (rCHO) cell cultivation. In this study, in two different recombinant CHO cell lines (DG44-origin), various components in typical serum-free media were evaluated for enhancement of the cell growth and the productivity of recombinant antibody, which is a first important step for the fortified medium development.

Materials and methods

Cell lines and media

The recombinant Chinese hamster ovary (rCHO)-A cell line expressing a chimeric antibody against the human platelet, designed for anti-thrombotic treatment was kindly provided from ISU Chemical Co. Ltd (Seoul, Korea). 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. Suspension adaptation of rCHO-A cell was performed in protein-free medium A supplemented with 1.00 μM of MTX.

The rCHO-B cell line expressing a chimeric antibody against the S surface antigen of Hepatitis B virus was selected at 1.00 μM MTX. Suspension adaptation of this CHO cell was performed in protein-free medium, 486F (WelGEN, Daegu, Korea).

Culture condition

Batch suspension culture was established in a 250 ml Erlenmeyer flask (Corning Corp., Corning, NY) with an inoculum of $2.0-3.0\times10^5$ cells ml^{-1} in working volume of 100 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.

Medium additives for nutrient fortification

Most of amino acids (Ajinomoto Co,. Inc., Tokyo, Japan) and vitamins (Sigma, St. Louis, MO) except for folic acid and riboflavin were dissolved at approximately 100-fold higher concentration in deionized water compared to the basal medium levels. Folic acid (Sigma, St. Louis, MO) and Riboflavin (Sigma, St. Louis, MO) were prepared in basal medium at final concentration. Insulin (Lilly, SA, France) and Long^{TM}R^{3}IGF-I (Gro*pep*, SA, Australia) were diluted in deionized water and hydrocortisone (Sigma, St. Louis, MO) was dissolved in pure ethanol. Other chemicals used were obtained from either Junsei Chemical Co. (Tokyo, Japan) or Sigma Chemical Co. in reagent grade and were dissolved in deionized water. All components were stored at −20 °C until used.

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 were analyzed 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 (A: pH 7.3, B: pH 1.3) was adopted to isolate the peak of immunoglobulin.

Amino acids were quantified by system Gold HPLC equipped with 166 UV detector (Beckman Instrument, San Ramon, CA) and C-18 column (Waters, Milford, MA) using FMOC derivatives. Buffer solution (A buffer) with 50 mM sodium acetate (pH 4.2, 80%) and acetonitrile (20%) and buffer solution (B buffer) with 50 mM sodium acetate (pH 4.2, 20%) and acetonitrile (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. The osmolality of cell culture media was generally between 295 and 305 mOsm Kg^{-1} and the pH between 7.30 and 7.40.

Results and discussion

Batch cultures of two rCHO cell lines in the basal protein-free media

Batch suspension cultures of two rCHO cell lines were carried out in the basal protein-free media. The growth profiles of two cell lines are shown in Figure 1. Cell density of rCHO-A cell line reached to 2.21×10^6 cells ml^{-1} at culture time of 172 h, and final antibody concentration was 82.50 arbitrary unit (AU) l^{-1} at culture time of 470 h (Figure 1(a), Table 1). The maximum specific growth rate at exponential growth phase and specific antibody productivity were determined as 0.42 day^{-1} and 3.59 AU$(10^6$ cells day$)^{-1}$, respectively, based upon the time courses in Figure 1(a). During the culture period, most of glucose was consumed and lactate concentration was maintained at approximately 0.70–0.80 g l^{-1}. Interestingly, after all of glucose was exhausted, lactate concentration also decreased and recombinant antibody was produced continuously. At the point of glucose depletion, the yield coefficient of antibody from glucose, $Y_{ab/glc}$, which is the relative amount of recombinant antibody produced from one gram of glucose, was obtained as 0.82 AU g^{-1} (Table 1).

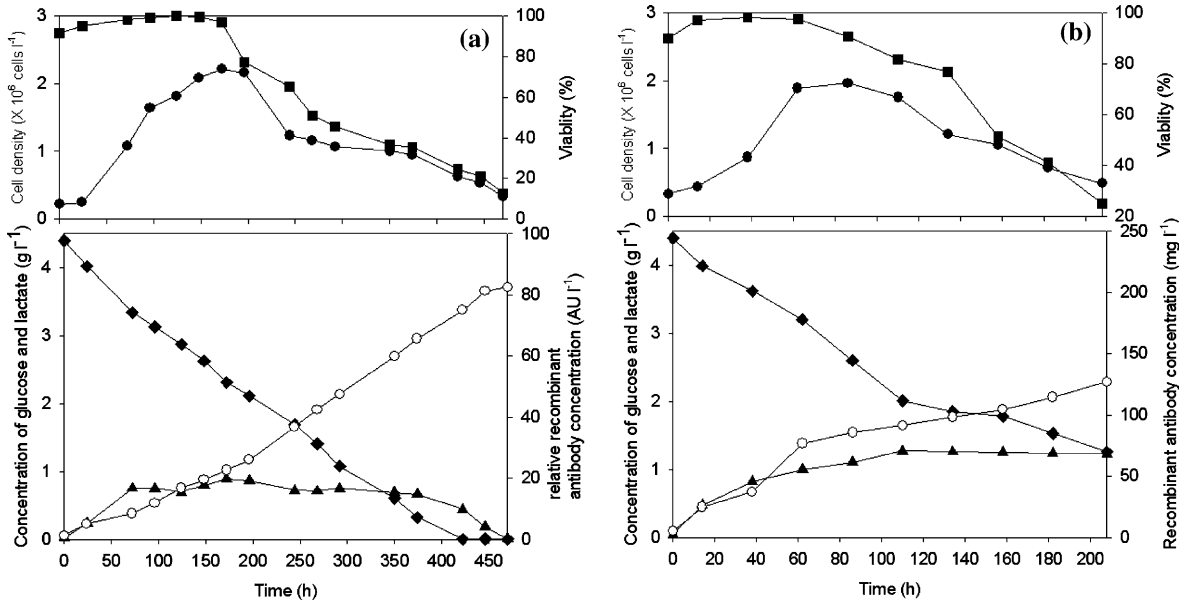

Figure 1. Cell growth and recombinant antibody production in basal media: (a) rCHO-A cell, (b) rCHO-B cell. Symbols are cell density (●), viability (■), glucose (◆), lactate (▲), and recombinant antibody (○).

Table 1. Quantitative comparison of rCHO-A cell in medium supplemented with various vitamins.

	rCHO-A cell line												
	C	BT	CP	CC	CB	FA	IT	NA	PN	RB	TH	AS	F + N
Maximum specific growth rate (day^{-1})	0.42	0.83	0.27	0.81	0.48	0.46	0.57	0.75	0.80	0.81	0.98	0.71	0.91
Maximum cell density (10^6 cells ml^{-1})	2.21	2.18	2.57	2.52	2.30	2.37	1.76	1.96	2.56	2.65	2.21	2.19	2.35
Final antibody concentration (AU l^{-1})	82.50	55.74	74.66	92.88	62.19	89.12	60.76	56.58	37.46	58.47	91.43	62.94	54.17
Integral of viable cell density (10^6 cells day)	22.99	21.44	21.99	25.84	24.82	29.37	16.19	21.18	23.82	24.08	23.71	20.79	19.82
Specific antibody productivity (AU(10^6 cells day)$^{-1}$)	3.59	2.60	3.39	3.59	2.50	3.03	3.75	2.67	1.57	2.43	3.86	3.02	2.73
$Y_{ab/glc}$(AU g^{-1})	0.82	0.73	0.85	0.90	0.80	0.79	0.82	0.74	0.70	0.76	0.91	0.83	0.75

C: basal medium; BT: Biotin; CP: D-calcium pantothenate; CC: choline chloride; CB: cyanocobalamin; FA: folic acid; IT: i-inositol; NA: niacinamide; PL: pyridoxal·HCl; PN: pyridoxine·HCl; RB: riboflavin; TH: thiamine·HCl; AS: ascorbic acid; F + N: folinic acid + niacinamide.

The growth profile of rCHO-B cell line differed from that obtained with the rCHO-A cell line. Cells grew to 1.97×10^6 cells ml^{-1} at culture time of 86 h with a maximum specific growth rate of 0.77 day^{-1} (Figure 1(b)). The final antibody concentration was 126.86 mg l^{-1} at culture time of 208 h (Table 2). During the culture period, while glucose concentration dropped to 1.22 g l^{-1}, lactate concentration was maintained at approximately 1.26 g l^{-1}. Specific antibody productivity and $Y_{ab/glc}$ were determined as 15.97 μg (10^6 cells day)$^{-1}$ and 0.040 g g^{-1} (gram antibody produced from gram glucose consumed), respectively Figure 1(b), Table 2)

Fortification of vitamins

Since it has been reported that vitamin depletion can limit cell growth and supplementation of some vitamins enhance either cell growth or recombinant antibody production (Stoll et al. 1996; Ducommun et al. 2001; Ishaque and Al-Rubeai 2002), it was decided to double the concentration of each vitamin in the basal protein-free medium to prevent any vitamin depletion. Thirteen water-soluble vitamins (D-biotin, D-calcium pantothenate, choline chloride, cyanocobalamine, folic acid, i-inositol, niacinamide, pyridoxal·HCl, pyridoxine·HCl, riboflavin, thiamine·HCl, ascorbic acid, folinic acid) present in the basal protein-free medium were considered, and their concentration was changed individually.

For rCHO-A cell line, with the supplementation of twelve water-soluble vitamins except for D-calcium pantothenate, the maximum specific growth rates were much higher than that obtained with the basal medium (Table 1). The pattern of lactate usage after complete depletion of glucose was observed in all cases (In our preliminary research, lactate usage has been observed in the cultures of various cell lines, depending on the culture conditions). With the fortification of choline chloride and folic acid, while specific antibody productivities (3.59 and 3.03 AU(10^6 cells day)$^{-1}$, respectively) were similar to that obtained from the basal protein-free medium, integrals of viable cell density (25.35 and 29.37 10^6 cells day, respectively) were higher than the control (Table 1), resulted in increased final antibody concentrations (92.88 and 89.12 AU l^{-1}, respectively). Also, when thiamine·HCl was fortified to the basal protein-free medium, recombinant antibody production (91.43 AU l^{-1}) was enhanced with a high specific antibody productivity of 3.86 AU(10^6 cells day)$^{-1}$ (Figure 2, Table 1).

In the culture of rCHO-B cell line, various vitamins had a positive effect on cell growth and recombinant antibody production. When choline chloride and i-inositol were fortified, rCHO cells grew to 2.46×10^6 cells ml^{-1} and 2.49×10^6 cells ml^{-1}, which were over 20% higher than the control. While the maximum specific growth rates were similar or slightly lower compared with that obtained in original basal medium, final antibody concentrations and $Y_{ab/glc}$ were increased since viable cell densities were maintained highly along the cultivation period with supplementation of choline chloride, i-inositol, niacinamide, pyridoxine·HCl, and thiamine·HCl. Especially, when thiamine·HCl was fortified, final antibody concentration and $Y_{ab/glc}$ were 172.37 mg l^{-1} and 0.058 g g^{-1}, which were 36% and 43% higher than those obtained with the basal

Table 2. Quantitative comparison of rCHO-B cell in medium supplemented with various vitamins.

	rCHO-B cell line													
	C	BT	CP	CC	CB	FA	IT	NA	PL	PN	RB	TH	AS	F + N
Maximum specific growth rate (day^{-1})	0.77	0.82	0.61	0.74	0.75	0.70	0.80	0.81	0.36	0.61	0.61	0.57	0.73	0.69
Maximum cell density (10^6 cells ml^{-1})	1.97	2.06	1.90	2.46	2.05	2.39	2.49	2.02	0.40	1.76	2.07	2.20	2.10	1.79
Final antibody concentration ($mg\,l^{-1}$)	126.86	110.33	115.62	131.22	122.11	116.35	158.35	159.21	26.54	145.00	107.56	172.37	105.67	104.38
Integral of viable cell density (10^6 cells day)	7.94	9.86	8.88	10.84	10.17	10.37	11.75	15.92	0.73	10.43	9.59	12.21	7.86	7.52
Specific antibody productivity (μg (10^6 cells day)$^{-1}$)	15.97	11.19	13.02	12.10	12.00	11.21	13.48	10.00	36.31	13.90	11.21	14.11	13.44	13.88
$Y_{ab/glc}$($g\,g^{-1}$)	0.040	0.038	0.043	0.044	0.043	0.041	0.054	0.044	0.021	0.043	0.038	0.058	0.041	0.041

C: basal medium; BT: Biotin; CP: D-calcium pantothenate; CC: choline chloride; CB: cyanocobalamin; FA: folic acid; IT: i-inositol; NA: niacinamide; PL: pyridoxal·HCl; PN: pyridoxine·HCl; RB: riboflavin; TH: thiamine·HCl; AS: ascorbic acid; F + N: folinic acid + niacinamide.

protein-free medium (Table 2, Figure 3). Previous researchers reported that these vitamins which are members of the vitamin B complex are known to play an important role to inhibit rapid apoptosis in mammalian cell culture. Deprivation of choline chloride (vitamin B_4) inhibits the phospholipid formation (Ishaque and Al-Rubeai 2002) and thiamine·HCl (vitamin B_1) could assist pyruvate to be metabolized rapidly and prevent the assimilation of toxic lead in animal cells (Pannunzio et al. 2000). Niacinamide (vitamin B_3) could enhance the growth capacity and prevent the apoptosis (Ishaque and Al-Rubeai 2002). Through these results, it is expected that choline chloride, i-inositol, niacinamide, pyridoxine·HCl, and thiamine·HCl have inhibitory effects on cell apoptosis in the cultivation rCHO-A or rCHO-B cells and they can be possible vitamin candidates for fortified medium development to enhance the cell growth and recombinant antibody production.

Peculiarly, when the pyridoxal·HCl was fortified, culture performances of two rCHO cells differed from those obtained with the supplementation of other vitamins (Table 2). The rCHO-A cells didn't grow at all and cell viability declined rapidly after 24 h, while rCHO-B cells only grew to 0.40×10^6 cells $m1^{-1}$ and cell viability decreased rapidly during 86 h of culture period. The specific antibody productivity of rCHO-B was 2.27 times higher than the control. These results showed that pyridoxal·HCl had an inhibitory effect on cell growth of two rCHO cells and it is recommended to control its concentration in the basal medium precisely for the enrichment of the cell growth and final antibody concentration.

Supplementation of amino acid

In our preliminary study, amino acid consumption or production profiles in the culture medium by rCHO cells in medium were investigated. After amino acid analysis of medium before and after cultivation, amino acids were divided into two groups: consuming amino acids and producing amino acids. The amino acids included in the consuming group were L-aspartic acid, L-arginine, L-cystine, L-histidine, L-leucine, L-lysine, L-methionine, L-serine, L-threonine, L-tyrosine, L-valine, and L-glutamine, while the amino acids contained in the producing group

were L-alanine, glycine, L-glutamate, L-isoleucine, L-proline, L-phenylalanine, and L-tryptophane.

Various preceding researches suggested that mammalian cell utilizes other amino acids as well as glutamine as energy sources for its survival and product formation. Methionine as a donor of sulfur and methyl groups is required in rCHO cell cultivation for high-level production of therapeutic protein. Serine was reported as a growth-limiting nutrient used in batch culture of CHO cells (Lee et al. 1999). As other research groups reported, with high ammonia concentration in the medium, remarkable increase of alanine production and aspartic acid consumption were observed (Lao and Toth 1997). Also, accumulated glutamate by the glutamine metabolism contributes to production of alanine through transaminase reactions (Lee et al. 1999). In hybridoma cultivation, several kinds of amino acid such as glutamine, asparagines, and glycine are known to prevent cellular apoptosis (Stoll et al. 1996; Ducommun et al. 2001). Appropriately balanced supplementation of amino acid was shown to be very efficient to increase viable cells and final concentration of recombinant antibody (Stoll et al. 1996; Ducommun et al. 2001).

Batch suspension cultures of two rCHO cell lines were carried out with the supplementation of consuming and producing amino acids to investigate their effect on cell growth and end-product formation. The concentrations of consuming and producing amino acids in basal medium were doubled. When the consuming amino acids were supplemented to the medium, during the exponential growth phase rCHO-A cells grew with a maximum specific growth rate of 0.36 day^{-1} and cell density reached to 2.08×10^6 cells ml^{-1} at culture time of 124 h. The final antibody concentration was 58.21 AU l^{-1} and specific antibody productivity was 2.68 AU $(10^6 \text{ cells day})^{-1}$, respectively. However, when the producing amino acids were added, maximum cell density, final antibody concentration, and specific antibody productivity were slightly higher than those obtained from the fortification of the consuming amino acids. It was against our expectation. Supplementation of the consuming or the producing amino acids did not enhance culture performances compared to the control Table 3).

In the cultures of rCHO-B cell line, when the producing amino acids were supplemented, there were no significant changes in cell density and antibody production. However, with the supplementation of consuming amino acids, cells grew to 1.99 cells ml^{-1} at culture time of 62 h only and specific growth rate was 0.77 day^{-1}. In particular, final antibody concentration and specific antibody productivity were increased upto 232.85 mg l^{-1} and 27.48 $\mu g(10^6 \text{ cells day})^{-1}$, respectively, which were 1.84 and 1.72 times higher than those obtained from the control. Also, $Y_{ab/glc}$ value of 0.060 g g^{-1} revealed 50% increase compared to that of the control, 0.040 g g^{-1} (Figure 4, Table 4). These results showed that a simple strategy of doubling the concentration of con-

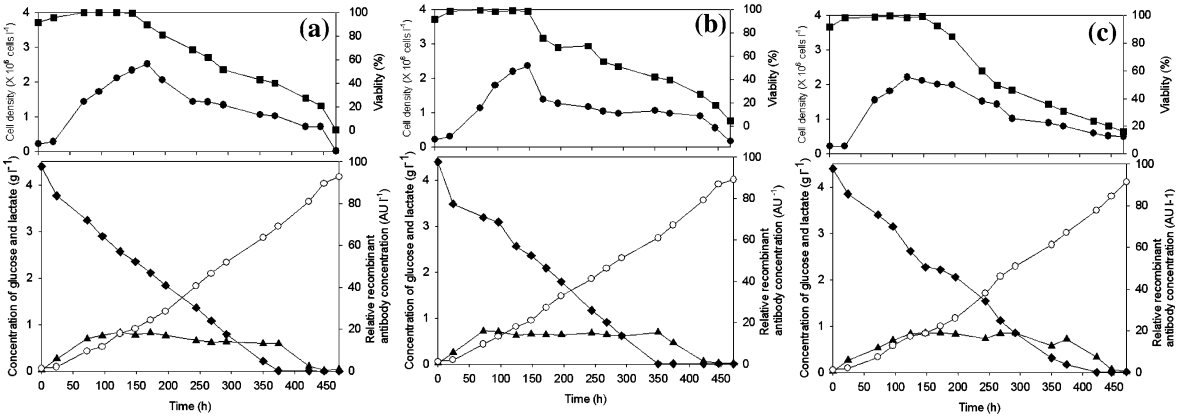

Figure 2. Effect of supplementation of vitamins on cell growth and recombinant antibody production in rCHO-A cell cultures: (a) choline chloride, (b) folic acid, (c) thiamine HCl. Symbols are cell density (●), viability (■), glucose (◆), lactate (▲), and recombinant antibody (○).

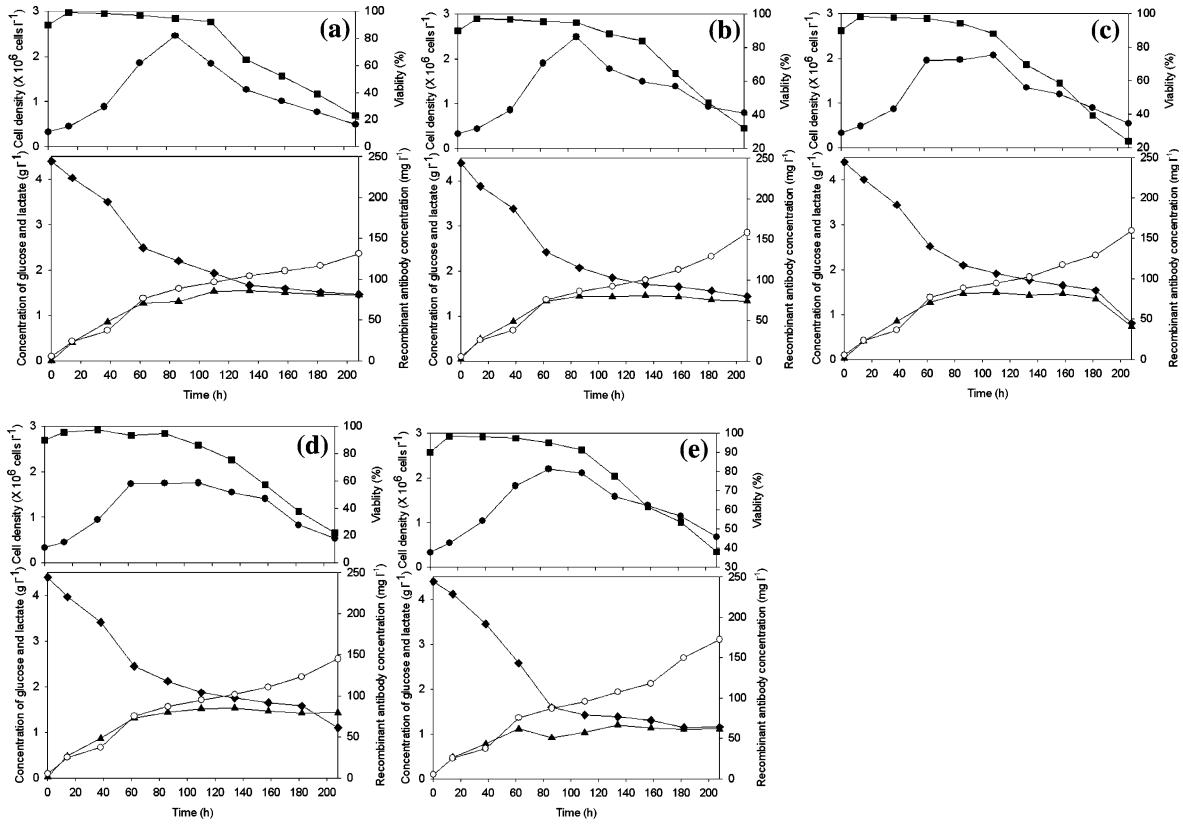

Figure 3. Effect of supplementation of vitamins on cell growth and recombinant antibody production in rCHO-B cell cultures: (a) choline chloride, (b) i-inositol, (c) niacinamide, (d) pyridoxine HCl, (e) thiamine HCl. Symbols are cell density (●), viability (■), glucose (◆), lactate (▲), and recombinant antibody (○).

suming amino acids is very efficient for production of recombinant antibodies from the rCHO-B cells.

Supplementation of hormones, inorganic salts, and other chemicals

Supplementation effect of various hormones, inorganic salts and other chemicals in rCHO cultures were evaluated. They were grouped by their metabolic functions, and their supplemented amounts were listed in Table 5.

The rCHO-A cells showed significantly increased specific growth rate, resulting in high maximum cell densities in most of the experimental cases except for cultures with β-mercaptoethanol, chloroquine, and inorganic salt group 2. Among all of experimental groups, Long-

Table 3. Quantitative comparison of rCHO-A cell in medium supplemented with consuming and producing amino acids.

	rCHO-A cell line		
	Basal medium	Consuming amino acid group	Producing amino acid group
Maximum specific growth rate (day^{-1})	0.42	0.36	0.76
Maximum cell density (10^6 cells ml^{-1})	2.21	2.08	3.01
Final antibody concentration (AU l^{-1})	82.50	58.21	58.95
Integral of viable cell density (10^6 cells day)	229.85	217.28	201.90
Specific antibody productivity (AU(10^6 cells day)$^{-1}$)	3.59	2.68	2.92
$Y_{ab/glc}$(AU g^{-1})	0.82	0.78	0.77

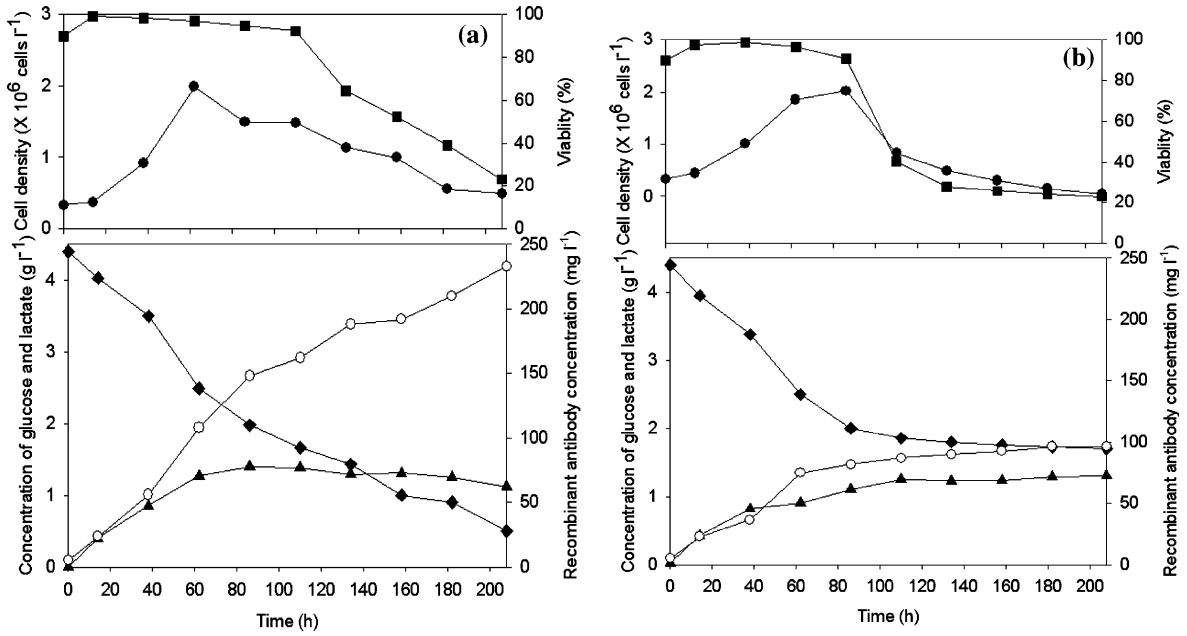

Figure 4. Effect of supplementation of consuming and producing amino acids on cell growth and recombinant antibody production in rCHO-B cell cultures: (a) consuming amino acid, (b) producing amino acid. Symbols are cell density (●), viability (■), glucose(◆), lactate (▲), and recombinant antibody (○).

$^{TM}R^{3}$IGF-I was a unique additive, showing positive effects on cell growth and antibody formation simultaneously (Figure 5(a)). Cells grew to 2.39×10^{6} cells ml^{-1} and final antibody concentration reached 88.60 AU l^{-1}. The integral of viable cell density was 22.25×10^{6} cells day and a specific antibody productivity was 3.98 AU$(10^{6}$ cells day$)^{-1}$ (Table 6).

The pattern of glucose exhaustion followed by lactate consumption was also observed in most of cultures using medium supplemented various chemicals. Significantly, the unique time course was shown with the supplementation of energy source group including glucose, sodium acetate, and sodium pyruvate (Figure 5(b)). When glucose concentration fell from 8.00 to 4.12 g l^{-1} in culture period of 470 h, lactate was produced gradually resulting in 1.52 g l^{-1} of final lactate concentration without any consumption. These results showed that lactate consumption by rCHO cells triggered by the depletion of glucose as a carbon sources and rCHO cell line utilized lactate as a substitutive carbon sources. Previous researches suggested that lactate dehydrogenase (LDH) catalyzing the conversion of lactate to pyruvate repressed lactate production (Lao and Toth 1997; Takagi et al.

Table 4. Quantitative comparison of rCHO-B cell in medium supplemented with consuming and producing amino acids.

	rCHO-B cell line		
	Basal medium	Consuming amino acid group	Producing amino acid group
Maximum specific growth rate (day^{-1})	0.77	0.77	0.61
Maximum cell density (10^{6} cells ml^{-1})	1.97	1.99	2.02
Final antibody concentration	126.86	232.85	96.25
Integral of viable cell density (10^{6} cells day)	7.94	8.47	6.78
Specific antibody productivity (μg $(10^{6}$ cells day$)^{-1}$)	15.97	27.48	14.20
$Y_{ab/glc}$ (g g^{-1})	0.040	0.060	0.036

Table 5. Various hormones, inorganic salts, and other compounds.

Group name	Additive supplements (concentration)
Hormones	Insulin
	Long^{TM}R^{3}IGF-1
	Triiodothyronine
	Hydrocortisone
Energy source group	*d*-glucose (4.4 g/L) + sodium acetate·3H2O (3.7 mM) + sodium pyruvate (1 mM)
Inorganic salt group 1	KCl (4.2 mM) + KNO_3 (16 nM) + $NaHCO_3$ (14 nM) + Na_2HPO_3 (0.5 mM) + $CuSO_4{\cdot}5H_2O$ (7.8 nM) + $FeSO_4$ (1 μM) + $MnSO_4{\cdot}H_2O$ (1 nM)
Inorganic salt group 2	Mo_7O_{24} (1 nM) + $NiCl_2$ (0.5 nM) + H_2SeO_3 (10 nM) + $NaSiO_3$ (10 μM) + $SnCl_2$ (0.5 nM)
Others	Putrescine (0.1 mM) + Pluronic F-68 (0.1%)
	β-mercaptoethanol (50 μM) + chloroquine (25 μM)

2001) and as a result, recombinant antibody was produced much more until all of lactate was exhausted completely.

For rCHO-B cell line, when insulin, Long-^{TM}R^{3}IGF-I, hydrocortisone, and inorganic salts were supplemented to the basal medium, the maximum specific growth rates were 0.94, 0.94, 0.84, and 0.84 day^{-1}, respectively (Table 6). Maintaining high viable cell density, with Long^{TM}R^{3}IGF-I and triiodothyronine (T_3), the final antibody concentrations were about 23% higher compared with the control, resulting in high levels of $Y_{ab/glc}$ (0.054 and 0.053 g g^{-1}, respectively). Putrescine and Pluronic F-68 were potential candidates for enhancing the antibody production. With the highest maximum cell density of 2.34×10^6 cells ml^{-1}, recombinant antibody of 186.71 mg l^{-1} was produced (Figure 6, Table 7).

Insulin as a regulator of glucose metabolism and transportation was reported a typical growth factor present in serum (Okamoto et al. 1996; Chen

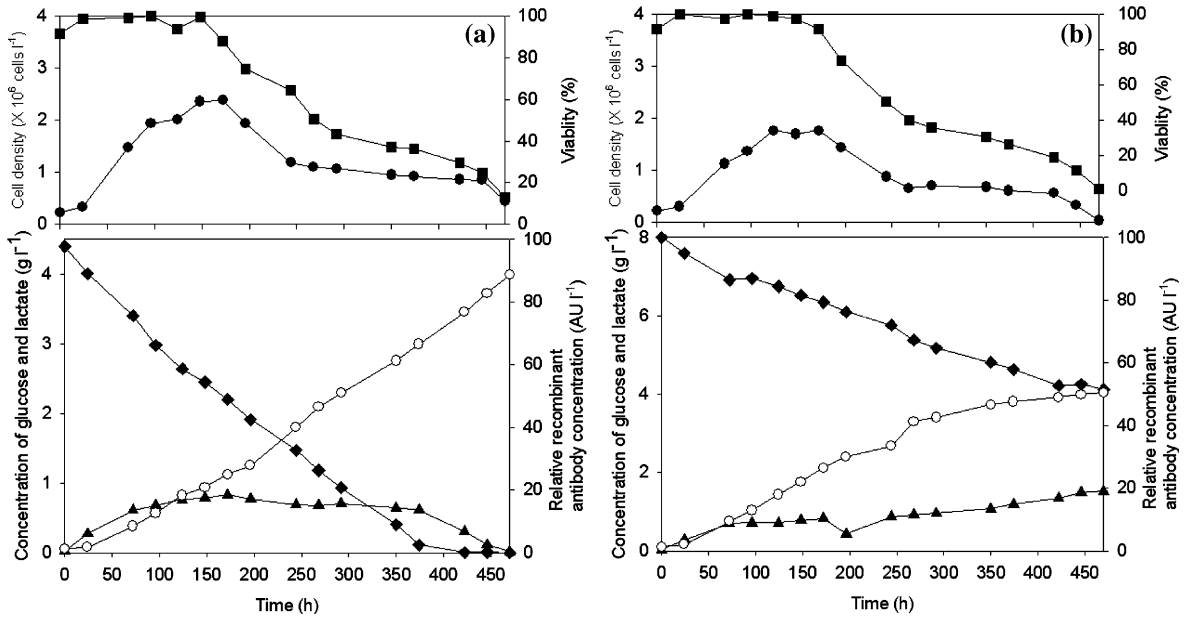

Figure 5. Effect of supplementation of various additives on cell growth and recombinant antibody production in rCHO-A cell cultures: (a) Long^{TM}R^{3}IGF-I, (b) energy source group. Symbols are cell density (●), viability (■), glucose (◆), lactate (▲), and recombinant antibody (○).

Table 6. Quantitative comparison of rCHO-A cell in medium supplemented with various hormones, inorganic salts, and other compounds.

	rCHO-A cell line									
	C	IS	LR	T_3	HC	P + P	M + C	ES	IS1	IS2
Maximum specific growth rate (day^{-1})	0.42	0.78	0.74	0.75	0.56	0.89	0.41	0.66	0.54	0.41
Maximum cell density (10^6 cells ml^{-1})	2.21	2.48	2.39	2.30	2.36	2.19	0.51	1.76	1.94	2.50
Final antibody concentration (AU l^{-1})	82.50	53.62	88.60	56.92	68.27	58.66	21.82	50.44	56.59	75.91
Integral of viable cell density (10^6 cells day)	22.99	19.93	22.25	26.69	25.32	25.27	17.70	20.40	23.25	22.37
Specific antibody productivity (AU(10^6 cells day)$^{-1}$)	3.59	2.69	3.98	2.13	2.69	2.32	1.23	2.47	2.43	3.39
$Y_{ab/glc}$(AU g^{-1})	0.82	0.70	0.80	0.74	0.88	0.77	0.61	0.74	0.76	0.78

C: basal medium; IS: Insulin; LR: Long^{TM}R^{3}IGF-1; T_3 : triiodothyronine; HC: hydrocortisone; P + P: putrescine + Pluronic F-68; M + C: β-mercaptoethanol + chloroquine; ES: energy source group; IS1: inorganic salt group 1, IS2: inorganic salt group 2

et al. 2000). From a viewpoint of designing a serum-free medium, the replacement of insulin by the synthetic compound is a very attractive task. Insulin-like fusion protein, Long^{TM}R^{3}IGF-I, was reported to show higher cell viability and expression level of therapeutic protein than a recombinant insulin (Morris and Schmid 2000). Triiodothyronine (T_3), thyroid hormone, plays an important role in induction of transcription factors in several mammalian cell cultures (Trentin and Alvarez-Silva 1998; Mercier et al. 2001). Putrescine was proved as an important supplement in the cultures of several mammalian cells including rCHO cell and Pluronic F-68, a typical surfactant in suspension culture, also showed a protective effect on cell from agitation damage (Kim 1998). Showing high specific growth rate, hydrocortisone is related to cell proliferation in various animal cell culture including several rCHO cell lines (Lee et al. 1999). Like previous reports, in the cultivation of rCHO-A or rCHO-B cells, Long^{TM}R^{3}IGF-I, triiodothyronine (T_3), putrescine and Pluronic F-68 showed positive effects on cell growth and antibody production and they can be selected as components for the enriched medium to improve the cell growth and recombinant antibody production.

Remarkably, although cell growth of two cell lines was restricted in the culture medium supplemented with β-mercaptoethanol and chloroquine, final antibody concentration and specific antibody productivity were relatively high compared with those obtained from the basal medium (Figure 6, Table 7).β-mercaptoethanol is an useful and usual component in serum-free media for embryos culture rather than rCHO cell culture (Geshi et al. 1999; Bagis and Mercan 2004) and chloroquine is famous for the therapeutic agent in the treatment of malaria (Ross et al. 2000). Their unacquainted effects on rCHO cell growth and product formation offers interesting assignments, which are worth studying.

Conclusion

Batch suspension cultures of two rCHO cell lines with supplementation of various medium components were performed in serum-free conditions. Like other research group's reports, although two different rCHO cell lines have the same origin, DG44, their growth and production profiles for recombinant antibody production showed large differences. Addition of choline chloride, folic acid, thiamine·HCl, and Long^{TM}R^{3}IGF-I had positive effects on cell growth and antibody production in rCHO-A cultures. In the cultivation of rCHO-B cell which was more sensitive to its environmental changes, increased concentrations of hormones such as Long^{TM}R^{3}IGF-I and triiodothyronine (T_3) as well as various vitamins including choline chloride, i-inositol, niacinamide, pyridoxine·HCl, and thiamine·HCl enhanced the culture performance. It was also shown that a simple strategy to double the concentration of consuming amino acids was very efficient for production of recombinant antibodies from the rCHO-B cells. Through selective combination of these effective components, we expect an improved recombinant antibody production in a synergic manner.

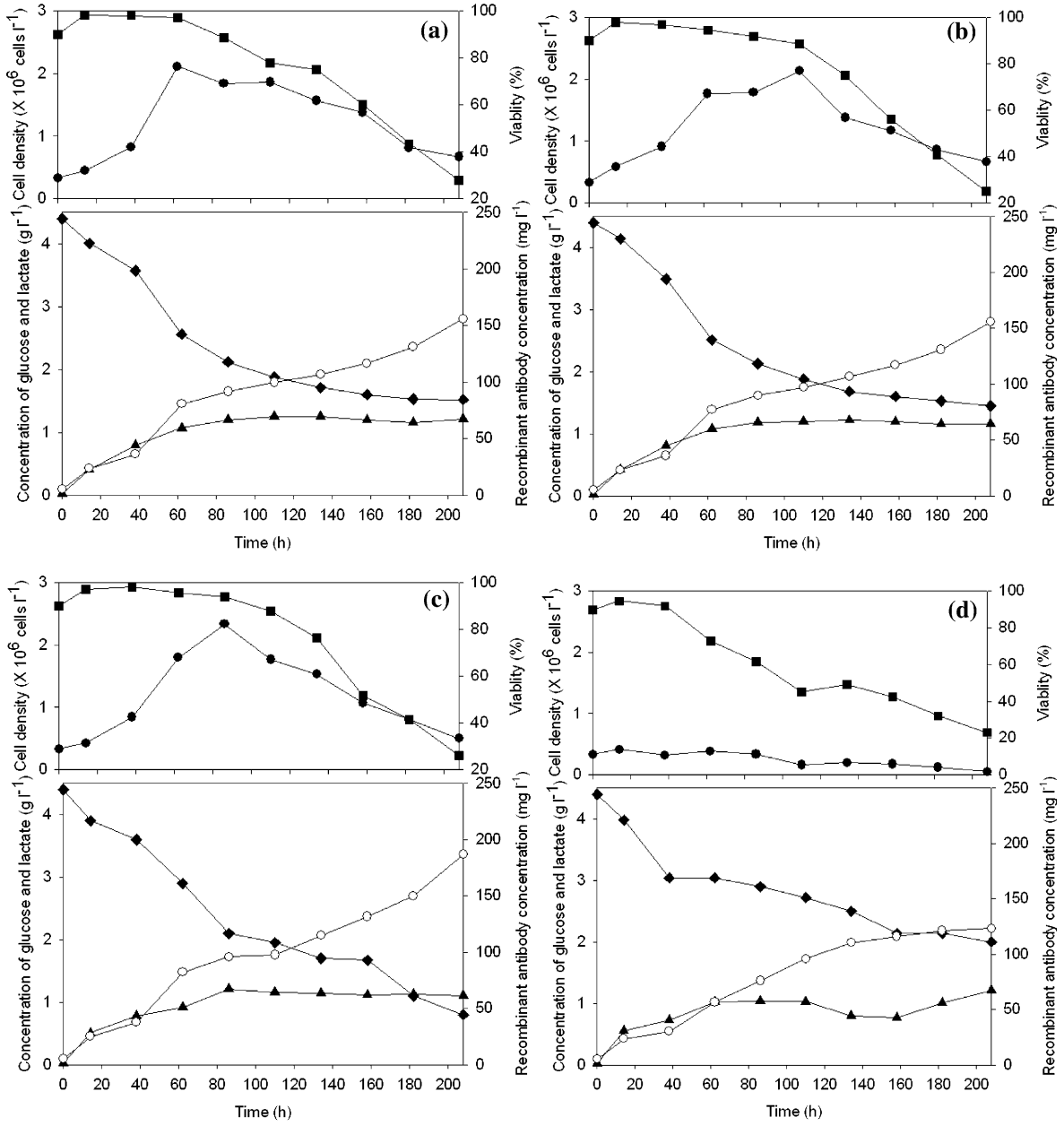

Figure 6. Effect of supplementation of various additives on cell growth and recombinant antibody production in rCHO-B cell cultures: (a) Long^{TM}R^{3}IGF-I, (b) triiodothyronine, (c) putrscine + Pluronic F68, (d) β-mercaptoethanol + chloroquine. Symbols are cell density (●), viability (■), glucose (◆), lactate (▲), and recombinant antibody (○).

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Table 7. Quantitative comparison of rCHO-B cell in medium supplemented with various hormones, inorganic salts, and other compounds.

	rCHO-B cell line									
	C	IS	LR	T_3	HC	P + P	M + C	ES	IS1	IS2
Maximum specific growth rate (day^{-1})	0.77	0.94	0.94	0.66	0.84	0.76	0.38	0.43	0.67	0.84
Maximum cell density ($\times 10^6$ cells l^{-1})	1.97	2.34	2.11	2.14	2.22	2.34	0.41	1.14	1.66	2.01
Final antibody concentration (mg l^{-1})	126.86	116.36	155.91	155.55	112.62	186.71	123.21	116.08	120.53	125.37
Integral of viable cell density (10^6 cells day)	7.94	9.96	11.25	10.70	10.16	10.67	1.83	4.87	8.18	10.15
Specific antibody productivity (μg (10^6 cells day)$^{-1}$)	15.97	11.68	13.85	14.54	11.08	17.49	67.09	23.83	14.74	12.35
$Y_{ab/glc}$(g g^{-1})	0.040	0.042	0.054	0.053	0.041	0.052	0.051	0.058	0.054	0.040

C: basal medium; IS: Insulin; LR: Long^{TM}R^{3}IGF-1; T_3 : triiodothyronine; HC: hydrocortisone; P + P: putrescine + Pluronic F-68; M + C: β-mercaptoethanol + chloroquine; ES: energy source group; IS1: inorganic salt group 1, IS2: inorganic salt group 2

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