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Effect of α-Ketoglutarate on Monoclonal Antibody Production of Hybridoma Cell Lines in Serum-Free and Serum-Containing Medium

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Abstract Process development and optimization for increase population growth and protein productivity in mammalian cell culture have been studied for many years. In this study, the behavior of hybridoma cells was investigated using six-well micro-titer plate systems with a working volume of 4 ml. Mouse hybridoma cell lines D2 and 2C83G2 were seeded in serum-free and serum-containing media and cultured for 8 days. α-Ketoglutarate is an integral component of the tricarboxylic acid (TCA) cycle and is produced from glutamine via glutamate. To study its effect on cell growth, metabolism, and monoclonal antibody (mAb) production, 2 mM α-ketoglutarate (pH 7.2) was added in both media at the beginning of the cultivation and in another set after 72 h. High cell density was observed in D2 cell culturing in serum-free medium, while 2C83G2 cell line showed high cell density in serum-containing medium. However, both cell lines cultured in serum-free medium gave viability above 70% when grown for 8 days. The supplement of 2 mM α-ketoglutarate supported cell growth and mAb production of both hybridoma cell lines in serum-free and serum-containing medium. The addition of α-ketoglutarate at the beginning of the batch cultivation gave better result in cell growth and mAb production as compared to α-ketoglutarate supplementation after 72 h. However, addition after 72 h was better than no

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addition at all. This indicates that α-ketoglutarate have a positive effect on production and release of antibody.

Keywords Antibody production · α-Ketoglutarate · Serum-free medium · Hybridoma

Introduction

The biotechnology and pharmaceutical industrial sectors are increasingly focusing on mammalian cell culture for bio-production of various therapeutics including antibodies, hormones, interferons, erythropoietin, clotting factor, immunoadhesins, and vaccines. The market for monoclonal antibodies (mAb) alone is expected to grow 30% a year [1]. This demand has provided a challenge for developing efficient and cost-effective processes for mAb production.

Culture media play an important role for animal cell cultivation. The choice of the culture media used depends on the type of cells. Most often, animal-derived serum is added to the medium in different concentrations depending on the individual cell line. The use of animal-derived serum has some disadvantages besides cost. For example, serum might contain viruses or prions like bovine spongiform encephalopathy (BSE), which results in risks of viral contamination. A further disadvantage is the lot-to-lot variability so that no consistent composition is guaranteed. The major disadvantage is the high protein content of this complex mixture so that purification of the desired product often gets more complex [2]. For these reasons, there is a growing demand for serum-free media [3], or protein-free, chemically defined medium [4]. The advantages of serum-free media are widely recognized. It provides a more defined, controlled cell culture environment and avoids animal welfare issues [5]. Initially, serum was replaced by some proteins such as albumin, transferrin, and insulin. More recently, it has been possible in many cases to develop chemically defined, protein-free media free of all animal components. Because different cell lines have different requirements and vary considerably from one cell line to another, design of a single serum-free formulation to act as a serum substitute suitable for the growth of all cell line is impossible. Combinations of standard basal media may be tested for best result in terms of good cell number and productivity at minimal serum level [6, 7]. In some cases, metabolic analysis may help in media design [8].

Another factor to be taken into consideration in designing media and feeds is the potentially toxic effect of accumulated catabolites such as ammonia and lactate. Glucose is one of the main carbon and energy sources for hybridoma cells. L-Glutamine is an essential amino acid that also provides a major energy source in mammalian cells. Whereas glucose and glutamine are fundamental nutrients for hybridoma growth, ammonium and lactate are products of cell metabolism that can act as inhibitors when their concentrations lead critical values. Besides, glutamine can be degraded and converted to ammonia as a by-product, which is toxic to the cell and can affect protein glycosylation and cell viability. Glutamine via glutamate is converted to α-ketoglutarate, an integral component of TCA cycle, and the excess carbon exiting the TCA cycle is converted to pyruvate via malate shunt [9–11]. Numerous methods to reduce ammonia concentration in cultivation broth have been developed. This includes substitution of glutamine by pyruvate [12], replacing glutamine by glutamate and 2-oxoglutarate [13], or using glutamine-free medium [14] or enhanced cell growth by supplementing with tripeptide Gly–Lys–Gly in the basal medium [15] From these results, we hypothesized that α-ketoglutarate (α-KG) supplemented in medium can enhance growth, prolong culture, and improve productivity. We then compared the growth

and mAb production of two mouse hybridoma cell lines in two different media, serum-free and serum-containing medium. A multi-well system, a useful scale down of larger scale reactor process optimization described by Girard et al. [16], was used in this experiment. Process relevant data and culture behavior from these experiments can provide the information for process refinement in the larger bench or pilot-scale bioreactor system.

Materials and Methods

Materials

Goat anti-isotype mouse immunoglubulins (heavy-chain-specific), alkaline phosphatase goat anti-mouse polyvalent immunoglobulins, *p*-nitrophenyl phosphate (PNPP), bovine serum albumin (BSA), reduced nicotinamide adenine (β-NADH), glutamate dehydrogenase, and trichloroacetic acid were bought from Sigma Chemical Co. (USA). α-Ketoglutarate was purchased from Fluka (USA). Cell culture media, Roswell Park Memorial Institute medium (RPMI 1640) with L-glutamine, hybridoma serum-free medium with L-glutamine, and fetal bovine serum (FBS) were from Gibco Invitrogen Corp.(Grand Island, NY, USA). All other chemicals were analytical grade.

Cell Lines

Two different murine (mouse–mouse) hybridoma cell line obtained from Dr. Oraprapai Gajanadana (BIOTEC, NSTDA Thailand) were used in this study. The first cell line is hybridoma cell line D2, which produces IgG2b monoclonal antibody against Gemini virus. The second cell line, 2C83G2 hybridoma, is a hybridoma cell line which produces IgG3 monoclonal antibody against *Listeria* spp. flagella. These cell lines were anchorage-independent and have different myeloma partner cell. From a previous study in our laboratory, D2 cell line, we observed, grows as a monolayer on the surface of the container and can be removed by physical force, whereas 2C83G2 cell line grows in suspension and is not attached to the surface of container.

Cell Culture

Stock cultures were maintained in RPMI 1640 with L-glutamine supplemented with 10% FBS. Base medium used for serum-free culture was hybridoma serum-free medium (HSF). This serum-free medium is a low-protein (20 μg/ml) as insulin and transferrin. α-Ketoglutarate (2 mM), pH 7.2 was added in RPMI and serum-free medium at initial (batch) and after 72 h (fed batch) of cultivation.

The adaptation to serum-free culture was carried out in a 25-cm^2 T-flask. The hybridoma cell lines were further grown in this serum-free medium for 2 weeks before seeding. The experiment was done in six-well plates which have an area of 9.6 cm^2/well. Each well was filled with 4 ml of six different media: (1) HSF, (2) HSF + 2 mM α-KG added at initial, (3) HSF + 2 mM α-KG added after 72 h, (4) RPMI with glutamine + 10% FBS, (5) RPMI with glutamine + 10% FBS+ 2 mM α-KG added at initial, and (6) RPMI with glutamine + 10% FBS+ 2 mM α-KG added after 72 h. Cell lines D2 and 2C83G2 were seeded in each well at a density of 1×10^4 viable cells per milliliter. Plates were incubated in a humidified atmosphere of 5% CO_2 in air at 37 °C. Samples were taken daily (one plate per day) until the decline phase (8 days). The experiment was conducted in duplicate.

Analytical Method

Cell and media were obtained from the multi-well plates. Cell density and viability were determined with (0.1% *w*/*v*) Trypan blue on a hemocytometer. Glucose consumption was measured by dinitrosalicylic acid method. Lactic acid production was determined by gas chromatography method modified from Rao [17]. Ammonia concentration was analyzed using enzymatic assay described by Bergmeyer [18]. Monoclonal antibody concentration was analyzed using an enzyme-linked immunosorbent assay. Each of the 96-well microplates (Corning, COSTAR, USA) was coated with goat anti-isotype mouse immunoglobulins (100 μl/ml) in coating buffer (0.05 M carbonate buffer, pH 9.6) at 4 °C overnight. Then, the plate was blocked with 2% BSA in washing buffer. The culture supernatant and standard mAb were added in duplicate to the plate and incubated for 1 h. Then, a secondary antibody, alkaline phosphatase which was conjugated with goat anti-mouse polyvalent immunoglobulins, was added and incubated for 1 h. This was followed by reaction with PNPP substrate solution for 30 min. The absorbance was measured at 405 nm using a 96-well microplate reader.

Results and Discussion

A comparison between the effects of α-KG-supplemented culture media as well as method of supplementation, initially like in a batch or that supplemented after 72 h like in fed-batch cultivation, required a comparison of kinetic parameters, and mAb production in different culture conditions were studied. This was with two cell lines, 2C83G2 and D2, in different culture media, serum-containing and serum-free medium.

Effect of Serum on Cell Growth and Metabolism

Serum has been an important component in animal cell cultures. Serum provides a rich source of hormones, growth factors, and trace elements that can promote cell growth. However, the composition of serum is variable and undefined, which leads to inconsistent growth and productivity. Nowadays, industry has an increasing desire to use media free of animal components for both economical and regulatory reasons. Thus, cells in serum-containing culture were observed and transferred to serum-free media [19–21]. To study the effect of serum on cell growth of two hybridoma cell lines, D2 and 2C83G2 were used. Both cell lines were adapted to culture in serum-free medium for 2 weeks before seeding. Cell growth was monitored by total cell count under hemocytometer with Trypan blue staining and reported in viable cell density and percent viability. The viable cell density of D2 and 2C83G2 cell line cultured in the two media was described in Figs. 1 and 2.

The result showed that D2 cell line gave high cell density when cultured in serum-free medium than in serum-containing medium (Fig. 1). The maximal viable cell density of this cell cultured in serum-free medium was two times greater than culture in serum containing medium (Table 1). Yield of viable cell density against glucose ($Y_{cell/glu}$), lactic acid production rate, ammonia production rate, and lactate yield on glucose ($Y_{lac/glu}$) of D2 cultured in serum-free medium were higher than cultured in serum-containing medium (Table 1). These calculated parameters revealed that D2 cell line has better growth and metabolism in serum-free medium than cultures in serum-containing medium.

In contrast, in the 2C83G2 cell line grown in serum-containing medium, RPMI, the viable cell density was more than two times higher than when this cell line was grown in

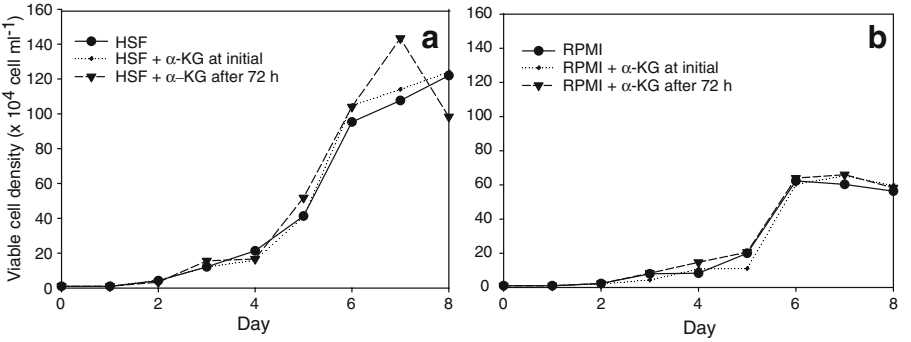

Fig. 1 Viable cell density of D2 cell line cultured on serum-free and serum-containing medium. **a** Viable cell density of D2 cell line cultured on HSF in different media conditions. **b** Viable cell density of D2 cell line cultured on RPMI + 10% FBS in different media conditions

HSF medium (Fig. 2). Growth parameters calculated are shown in Table 2. The specific cell growth rate (μ) of this cell line cultured in serum-containing medium (0.68 day^{-1}) was higher than this cell when cultured in serum-free medium (0.44 day^{-1}). Even glucose consumption rate of cell cultured in RPMI medium was less than glucose consumption rate of cell cultured in HSF, but yield ($Y_{cell/glu}$) of cell cultured in RPMI was 17.67, which was more than ten times higher than cell cultures in HSF medium (1.43). These data revealed that 2C83G2 cell lines had better growth in serum-containing medium than in serum-free medium. Indeed, these two cell lines have different behavior on being cultured because of different partner cell (myeloma). It turns out that cell lines are quite fastidious in their growth requirements and that such requirements vary considerably from one cell line to another. Therefore, it has not been possible to design a single serum-free formulation to act as serum substitute suitable for the growth of all cell lines. In fact, it has been shown that even different clones of CHO cells may require different formulations for optimal growth [8]. So, such comparison experiments may continue to be needed.

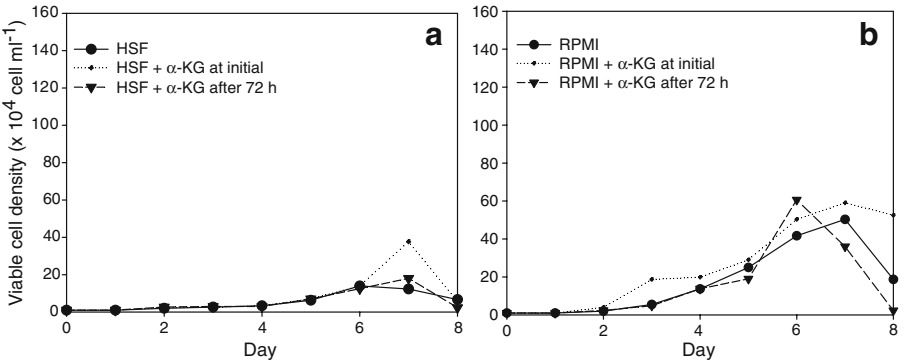

Fig. 2 Viable cell density and viability of 2C83G2 cell line cultured on serum-free and serum-containing medium. **a** Viable cell density of 2C83G2 cell line cultured on HSF in different media conditions. **b** Viable cell density of 2C83G2 cell line cultured on RPMI + 10% FBS in different media conditions

Table 1 Effect of α-ketoglutarate on cell growth and mAb production of D2 cell line in HSF and RPMI medium.

Kinetics	Medium conditions					
	HSF			RPMI		
	I	II	III	I	II	III
Maximum viable cell density ($\times 10^4$ cell ml^{-1})	122	143	124	62.30	65.30	66.00
Specific cell growth rate (day^{-1})	0.67	0.68	0.69	0.67	0.62	0.62
Doubling time (day)	1.03	1.02	1.00	1.03	1.13	1.12
Glucose consumption rate (mM day^{-1})	2.10	1.45	1.80	3.12	1.45	1.44
Yield of viable cell density against glucose (10^4 viable cell/mM glucose)	10.40	16.47	13.94	7.58	8.93	8.65
Lactate production rate (mM day^{-1})	7.80	5.52	4.04	3.32	2.58	2.58
Ammonia production rate (ng ml^{-1} day^{-1})	14.94	14.93	27.74	6.4	11.65	40.79
Lactate yield on glucose (mM lactate/mM glucose)	6.62	2.28	1.91	1.56	1.35	1.69

Each value represented the average data; the experiment was done in duplicate.

I = control (basal medium), II = basal medium + 2 mM α-ketoglutarate at initial, III = basal medium + 2 mM α-ketoglutarate after 72 h

Effect of α-Ketoglutarate Supplementation on Cell Growth and Metabolism

2C83G2 and D2 cell line, which were taken from stock culture at mid-exponential phase of growth, were inoculated into different conditions of culture media. HSF medium and serum-containing medium (RPMI 1640 + 10% FBS) were supplemented with 2 mM α-KG at the beginning and after cultivation for 72 h. Viable cell density of both cell lines in different media conditions is shown in Fig. 1.

The results revealed that the viability of both cell lines cultured in serum-free medium for 8 days was still above 70%, whereas for cell cultured in serum-containing medium,

Table 2 Effect of α-ketoglutarate on cell growth and mAb production of 2C83G2 cell line in HSF and RPMI medium.

Kinetics	Medium conditions					
	HSF			RPMI		
	I	II	III	I	II	III
Maximum viable cell density ($\times 10^4$ cell ml^{-1})	14	38	15	50.3	59	60.7
Specific cell growth rate (day^{-1})	0.44	0.56	0.45	0.68	0.67	0.67
Doubling time (day)	1.57	1.25	1.54	1.03	1.03	1.04
Glucose consumption rate (mM day^{-1})	1.15	1.42	0.85	0.48	1.03	0.68
Yield of viable cell density against glucose (10^4 viable cell/mM glucose)	1.44	1.66	1.66	17.67	13.56	16.74
Lactate production rate (mM day^{-1})	1.41	0.90	0.72	1.52	1.57	1.49
Ammonia production rate (ng ml^{-1} day^{-1})	6.34	7.24	9.28	9.83	13.71	19.68
Lactate yield on glucose (mM lactate/mM glucose)	0.76	0.86	0.93	2.45	1.67	2.29

Each value represented the average data; the experiment was done in duplicate.

I = control (basal medium), II = basal medium + 2 mM α-ketoglutarate at initial, III = basal medium + 2 mM α-ketoglutarate after 72 h

viability dropped to 20–60%. This result was different from experiments carried out by Ozturk et al. [6]. They observed that cell viability drops for the culture containing low serum, and serum helps to maintain the culture viability. Cell density of D2 cell line in HSF medium was greater than in RPMI medium, whereas in 2C83G2 cell line, RPMI medium with 10% FBS was suitable for cell growth than HSF medium. Also, media supplemented with α-KG had an effect on viable cell density. D2 cell line cultured in HSF with α-KG added after 72 h had higher cell density than HSF with initially added α-KG and control (no α-KG). However, with cell line cultures in RPMI medium, there was no difference in viable cell density for all three conditions (Fig. 1). In contrast, the α-KG supplement is affected on cell growth and gave higher cell density than control in both media for 2C83G2 cell line (Fig. 2).

Fed-batch cultivation is normally applied for improving cell culture longevity and final mAb titer. In our experiment, we decided to add α-KG after 72 h like fed-batch culture to prolong culture and increase productivity. However, the result showed that cell density did not increase much. In contrast, in D2 cell line, α-KG supplementation at initial phase in HSF medium like batch culture showed higher cell density than other conditions. Maximum viable cell density of D2 cell lines showed highest cell density in HSF medium with α-KG supplementation at initial (1.43×10^6 cell per milliliter), whereas for α-KG supplement in RPMI of this cell lines, maximum viable cell density was not different in α-KG supplementation in batch and fed-batch culture as shown in Table 1. For 2C83G2 cell line as well, the α-KG supplement, initially, in HSF medium showed high cell density, while α-KG supplement in RPMI media increased the viable cell density in both supplements, initially and after 72 h. From these results, it could be concluded that α-KG supplementation in serum-free medium and serum-containing medium can enhance and increase cell density, and it is better to add α-KG initially in the batch-type process.

Glucose consumption, lactic acid, and ammonia production of D2 and 2C83G2 cell lines were shown in Figs. 3 and 4, respectively. The calculated parameters to describe kinetics of cells in each media condition were also shown in Tables 1 and 2. The media used in our experiments had different compositions. HSF medium contains 20 mM glucose, while RPMI contains 12 mM glucose. Both media contain L-glutamine, with RPMI medium containing 2 mM L-glutamine, while the amount of L-glutamine remains confidential (propriety) in HSF medium. Tables 1 and 2 reveal that D2 and 2C83G2 cells cultured in serum-free medium exhibited slightly higher glucose utilization rates than serum-containing medium. Yield of viable cell density against glucose ($Y_{x/s}$) was highest in media with α-KG supplementation at initial stage in both cell lines. Also, the glucose consumption rate and specific lactate production rate was reduced in α-KG supplementation media condition. Lactate production rate of D2 cell line culture in both media was decreased from control condition, while rate was nearly similar in 2C83G2 cells in RPMI medium conditions. The theoretical maximum value for lactate yield on glucose ($Y_{lac/glu}$) in batch cultivation is 2. From our data, $Y_{lac/glu}$ of both cell lines in serum-free medium were higher than theoretical. This can be explained by taking into account glutamine metabolism, which can reconvert to pyruvate and lactate. Furthermore, our data are consistent with the research by Neermann et al. [22] who reported that the specific molar lactated/glucose ratios was higher in cells cultured under serum-free conditions compared to those cultivated in serum-supplemented medium.

Figures 3 and 4 show that the amount of ammonia production in media containing α-KG was higher than other media condition. The final ammonia concentration of D2 cell line in HSF with added α-KG after 72 h was 188 ng ml^{-1}, followed by 132 and 105 ng ml^{-1} in HSF with α-KG added initially and control conditions, respectively (Fig. 3a). Compared

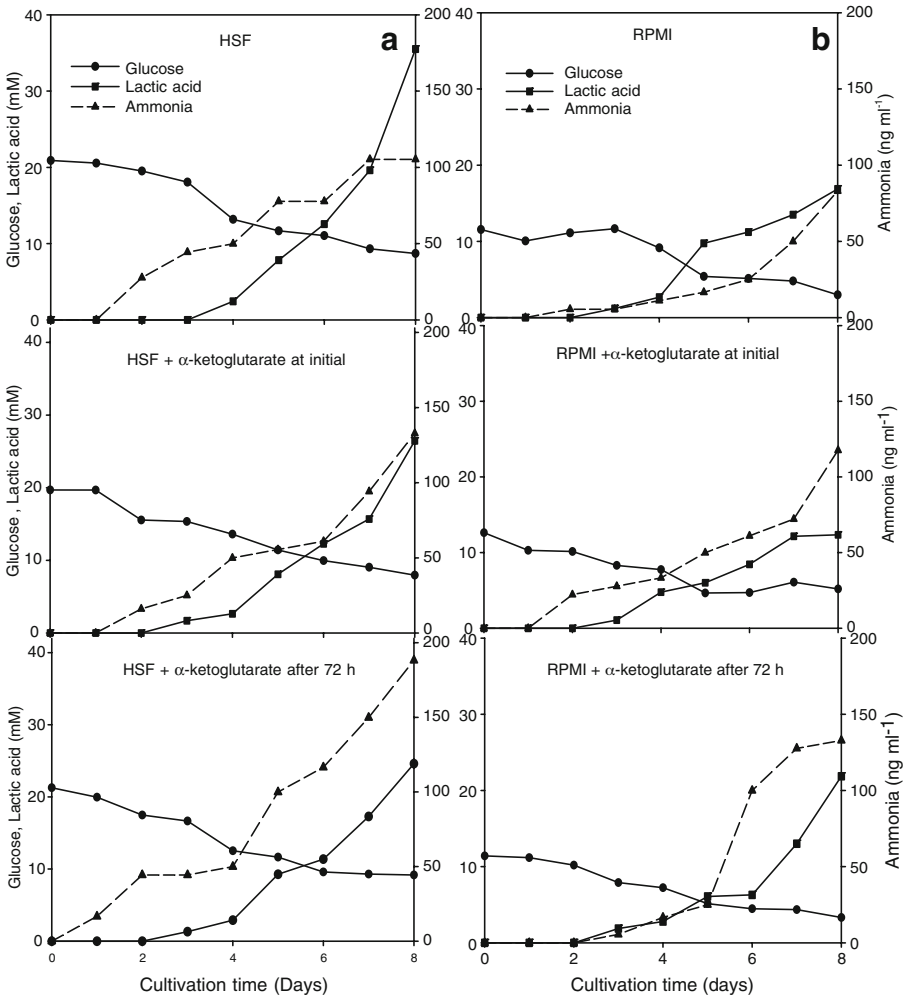

Fig. 3 Growth parameters of D2 cell line in HSF and RPMI media

with D2 cells cultured in RPMI medium, the final ammonia concentration of RPMI with added α-KG after 72 h was 133 ng ml^{-1} (Fig. 3b), which was less than the ammonia generated in HSF medium. In the same way, the 2C83G2 cell line supplemented with α-KG after 72 h in both media generated higher ammonia concentration than other medium conditions (Fig. 4). The amount of ammonia production of 2C83G2 cell line was less than the ammonia production by D2 cell line. It is clear that α-KG supplementation after 72 h in serum-free and serum-containing medium affected ammonia production by rapid increase after adding in both cell lines. There have been reports that concentration of ammonia or ammonium as low as 2–3 mM can reduce cell growth [23]. In our experiments, the maximal ammonia concentration was 188 ng ml^{-1} or equal to 0.011 mM, which was not toxic. This explains the fact that even though ammonia accumulated in system by affect of α-KG supplementation, the percent viability of cells in our result remains above 70% in HSF media.

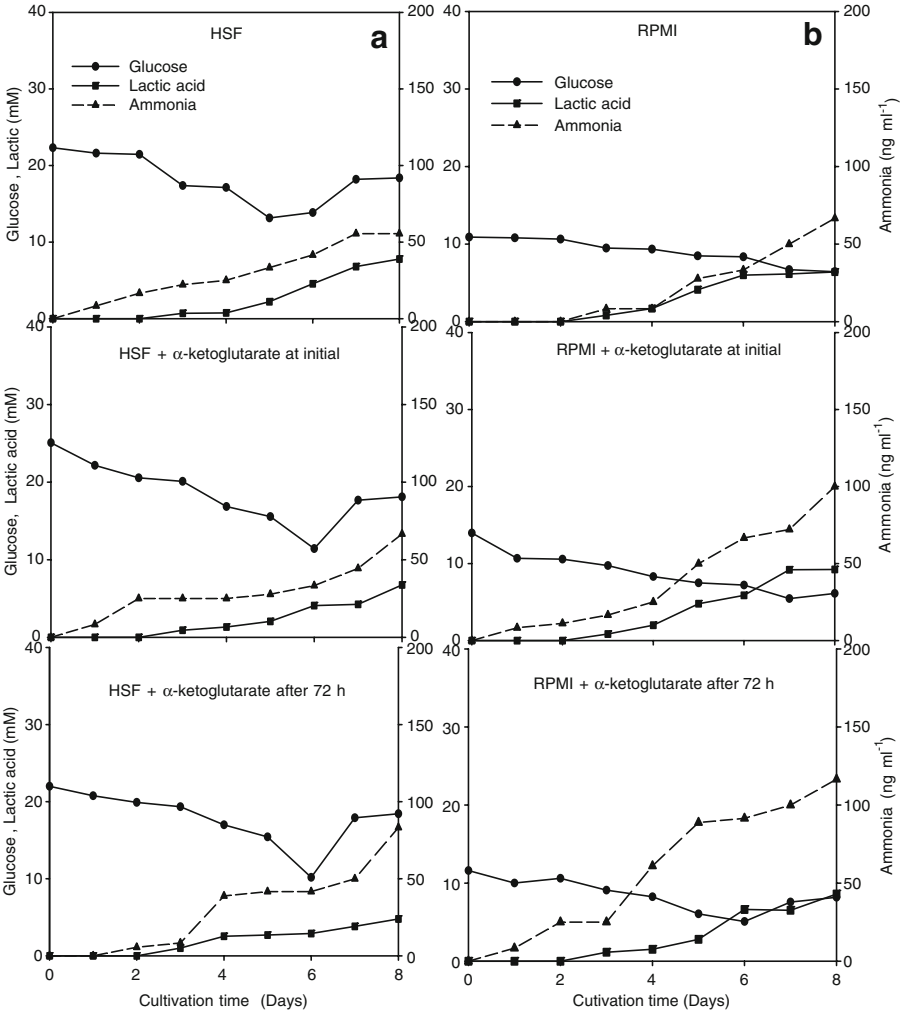

Fig. 4 Growth parameters of 2C83G2 cell line in HSF and RPMI media

The cellular degradation of glutamine has been intensively studied [24, 25]. Glutamine catabolism takes place mainly in the mitochondrial matrix. The phenomenon of α-KG supplement in media leading to an increase the ammonia production may be because the five carbon atom of α-KG cannot directly enter the mitochondria. α-KG must first transaminase to glutamate which can freely enter through mitochondria membranes and then transaminase from glutamate to α-KG again in the mitochondria matrix. This glutamate shuttle liberates one ammonium molecule [24]. The α-KG subsequently enters the TCA cycle and is completely oxidized to CO_2 or partly oxidized to aspartate and pyruvate via oxaloacetate and malate, respectively. Complete glutamine catabolism liberates two molecules of ammonia. Thus, the supplementation of α-KG in glutamine-containing media is increased ammonia in the system. The comparison of viable cell density results show that α-KG supplementation initially affect on cell density more than α-

KG supplemented after 72 h. This result indicates that α-KG not only prolongs culture but also leads to higher ammonia accumulation.

Effect of Serum on mAb Production

Figure 5 shows that the antibody yields were different in both cell lines which were cultured in serum-free and serum-containing medium. D2 cell line showed high mAb production in serum-free medium (Fig. 5a and b). In contrast, 2C83G2 cell line showed higher mAb production when cultured in serum-containing medium than in serum-free medium (Fig. 5c and d). Both cell lines follow the same pattern of mAb production with rapid increase in production of mAb on day 5. Cell density study in Fig. 2 showed that the number of cells also increase after 5 days of culture. This means that both cell lines produce antibody like a growth-associated product. However, the antibody productivity is significantly different between the different cell line with respect to the level of productivity as well as to the influence of culture condition [26]. Serum seems to have a regulatory as well as a simulative effect on the antibody productivity of 2C83G2 cell line. In contrast, D2 cell line showed higher mAb production when grown in HSF medium than this cell grown in serum-containing medium. This indicates that the formulation of HSF medium is suitable for D2 cell line to produce mAb more than RPMI + 10% FBS medium. It is known that serum-free medium is usually specific for a specific cell type and no single medium is suitable for all cell lines or even clones derived from the same parent cell line [27, 28]

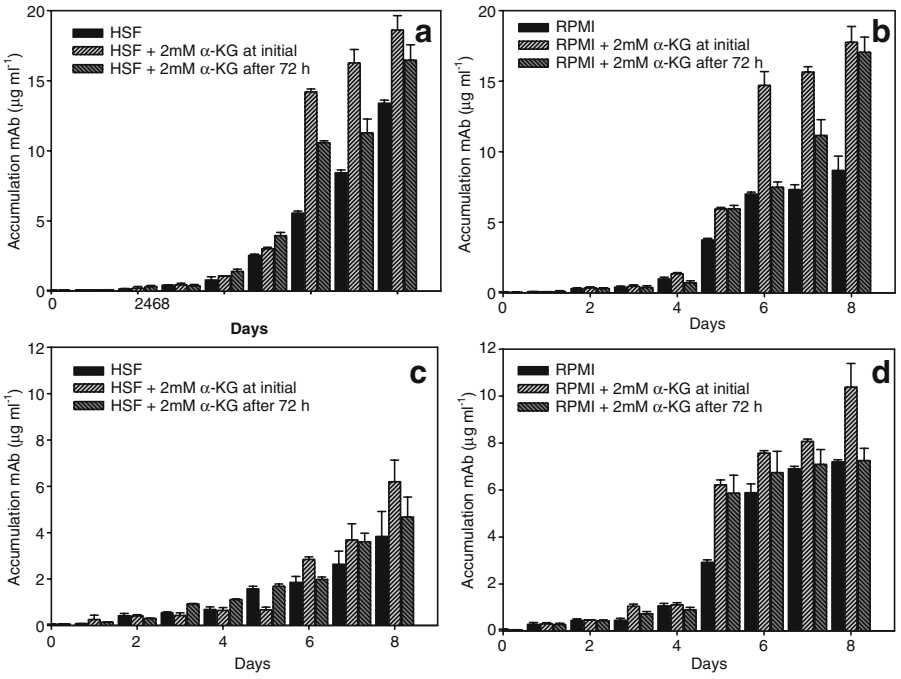

Fig. 5 mAb production at different time interval. **a** D2 cell line in HSF medium. **b** D2 cell line in RPMI medium. **c** 2C83G2 cell line in HSF medium. **d** 2C83G2 cell line in RPMI medium

Table 3 Effect of α-ketoglutarate on mAb production of D2 cell line.

D2 cell line	HSF			RPMI		
	I	II	III	I	II	III
Maximal mAb concentration (μg ml^{-1})	13.40	18.62	16.47	8.68	17.76	17.05
mAb production rate (μg ml^{-1} day^{-1})	2.94	4.88	4.59	2.22	4.73	2.66
Overall productivity (μg $ml^{-1}day^{-1}$)	1.68	2.31	2.08	1.09	2.22	2.13

Each value represented the average data; the experiment was done in duplicate.

I = control (basal medium), II = basal medium + 2 mM α-KG at initial, III = basal medium + 2 mM α-KG after 72 h

Effect of α-Ketoglutarate Supplement on mAb Production

The results shown in Fig. 5 clearly indicate that α-KG showed affect on mAb production in both cell lines in two different media. The supplementation of α-KG added initially gave higher mAb concentration than α-KG supplemented after 72 h and control medium, respectively. The calculated kinetic parameters of D2 cell line is reported in Table 3. The maximal mAb concentration of D2 cell cultured in HSF with added α-KG initially gave 18.62 μg ml^{-1}, followed by HSF with added α-KG after 72 h (16.47 μg ml^{-1}) and HSF alone (13.40 μg ml^{-1}). In the same cell line, when cultured in serum-containing medium, the maximal mAb concentration was two times higher in medium supplemented with α-KG than control condition. This result revealed that the α-KG supplemented in both media can enhance mAb production for D2 cell line, and it is better to supplement initially.

In the case of 2C83G2 cell line, result from Table 4 revealed that the maximal mAb concentration in HSF with added α-KG initially (6.20 μg ml^{-1}) was approximately two times higher than control condition (3.84 μg ml^{-1}). However, this cell line culture in RPMI supplemented with α-KG after 72 h is not significantly different on the maximal mAb concentration and overall productivity from control (no α-KG). Comparison of viable cell density of 2C83G2 cell lines (Fig. 2) in serum-containing medium supplementation with α-KG was higher than normal medium. This means that α-KG supplementation did not clearly induce on cell growth and mAb production for 2C83G2 cell line cultured in serum-containing medium. This result was explained by Jayme et al. [29] who reported that the additional nutrient medium optimization may be achieved by recognizing that cellular proliferation and biological production are often independent or competing activities. The medium formulation which supports maximal proliferation may be a poorer nutrient

Table 4 Effect of α-ketoglutarate on mAb production of 2C83G2 cell line.

2C83G2 cell line	HSF			RPMI		
	I	II	III	I	II	III
Maximal mAb concentration (μg ml^{-1})	3.84	6.20	4.68	7.20	10.38	7.25
mAb production rate (μg ml^{-1} day^{-1})	0.61	1.13	0.78	2.05	2.22	1.94
Overall productivity (μg ml^{-1} day^{-1})	0.48	0.78	0.59	0.9	1.3	0.9

Each value represented the average data; the experiment was done in duplicate.

I = control (basal medium), II = basal medium + 2 mM α-KG at initial, III = basal medium + 2 mM α-KG after 72 h

mixture for biological production. Hence, supplementing α-KG at initial and after 72 h gave the qualitative difference in serum-free and serum-containing medium may be because of the associated cell cycle, may relate to allocation of metabolic energy to support proliferate versus synthetic events, or may result from different nutritional building blocks required for these two cell lines. However, the data from Table 3 revealed that α-KG supplementation initially can give higher mAb production rate and overall productivity in both serum-free and serum-containing medium, which is definitely a positive outcome of this study.

Conclusions

From the results obtained in this study, we can conclude that the different cell lines have different behavior for mAb production when cultured in different media conditions. D2 cell line develops better culture growth in HSF medium than 2C83G2 cell line. It means that the different partner cell affects on growth and mAb production. The α-KG supplementation in both serum-free and serum-containing medium can increase the viable cell density and mAb production. α-KG supplement added initially like in batch cultivation and supplement after 72 h like fed-batch culture showed different results in mAb production. α-KG supplemented initially not only increased viable cells but also increased mAb production rate. In contrast, α-KG supplement like in fed-batch culture did not show effect on mAb production and also had high accumulation of ammonia. However, ammonia accumulations in our laboratory experiments were not high and were not toxic to cells. But in large-scale processes, the accumulation of ammonia may be a concern. Nevertheless, supplementation of α-KG can improve culture in terms of longevity and improve productivity. Further investigations of the amount of α-KG supplementation or substitute glutamine by α-KG in media for large-scale animal cell cultivation need to be done.

The selection of media for cultivation hybridoma cell line not only depends on type of cell line but also depends on behavior of cells as regards secretion of antibody. However, the advantages of using serum-free medium are costs, lot-to-lot stability, and absence of virus contamination which can come from serum. Overall, the results of these experiments suggested that using HSF medium can save production cost, and α-KG supplementation can enhance cell growth and mAb production.

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