ORIGINAL RESEARCH

Influence of different ammonium, lactate and glutamine concentrations on CCO cell growth

Igor Slivac · Višnja Blajić · Kristina Radošević ·
Zlatko Kniewald · Višnja Gaurina Srček

Abstract   In this study the effects of ammonium and lactate on a culture of channel catfish ovary (CCO) cells were examined. We also made investigation on the influence of glutamine, since our previous research revealed that this amino acid stimulated CCO cell growth more than glucose in a concentration-dependent manner. The effect of ammonium in cell culture included the considerable decrease in cell growth rate with eventual growth arrest as well as the retardation of glucose consumption. At ammonium concentrations above 2.5 mM, the cells displayed specific morphological changes. The effect of lactate was different to that of ammonium since the cell growth rate was progressively decreasing with the increase of lactate concentration, whereas the glucose consumption rate remained almost unchanged. Besides that, it was found that lactate was steadily eliminated from the culture medium when its initial concentration was relatively high. The influence of glutamine on CCO cell propagation showed that nutrient requirements of this cell line were mainly dependent on glutamine rather than glucose. The increase in glutamine concentration led to the increase in cell growth rate and consequent ammonia accumulation while the glucose utilization and lactate production were reduced. Without glutamine in culture medium cell growth was arrested. However, the lack of glucose reversed the stimulating effect of glutamine by decreasing cell growth rate and affecting amino acid utilization.

Keywords   Ammonium · CCO cells · Glutamine · Lactate · Metabolism

Introduction

The CCO cell line was derived from the ovaries of juvenile channel catfish more than 30 years ago (Bowser and Plumb 1980a). Since then these cells have been used for small-scale processes for channel catfish virus vaccine production (Bowser and Plumb 1980b) and related in vitro laboratory research (Stingley and Gray 2000). However the rising interest in analysis of toxic effects of various pharmaceutical and chemical agents accumulated in natural aquatic environment requires an increase in number of fish cell lines available for systematic and routine toxicology pre-investigation, prior to use of in vivo methods (Tan et al. 2008).

It may be presumed that the CCO cells have a significantly different metabolism regulation from the one taking place in the cells of their origin. This is a

I. Slivac · V. Blajić · K. Radošević · Z. Kniewald ·
V. Gaurina Srček (✉)
Laboratory of Cell Culture Technology and
Biotransformation, Faculty of Food Technology and
Biotechnology, University of Zagreb, 6 Pierotti St.,
10000 Zagreb, Croatia
e-mail: vgaurina@pbf.hr

common phenomenon demonstrated by the majority of cell lines currently used, and is a consequence of the changed growth conditions and the genetic alterations occurring during the immortalization process, leading to the establishment of a standard cell line. The metabolic deregulation is characterized by a very high and inefficient consumption of the main carbon, nitrogen, and energy sources, glucose and glutamine, and leads to the generation of metabolic waste-products, mainly lactate, ammonia, and some amino acids, like alanine or proline. It should be pointed out that this pattern is specific for batch culture system which is most often used in laboratory investigation and small-scale processes because of its simplicity. In this system cells are exposed to elevated glucose and glutamine levels contained in the culture medium and they regularely consume these nutrients at a rate much higher than what would be required to maintain cell growth.

In an effort to understand the toxicity of lactate itself, the effect of lactate addition to culture on the initial cell growth rate has been extensively studied (Glacken et al. 1988; Cruz et al. 2000). It is assumed that the change in extracellular pH and osmolality as a result of lactate production are considered responsible for cell growth inhibition (Omasa et al. 1992; Patel et al. 2000). The toxic effect of ammonia is evident at concentrations approximately one order of magnitude lower than the toxic effect of lactate, for a variety of cell lines (Mirabet et al. 1997; Newland et al. 1994). It appears that the ammonia level which retards cell growth depends on the cell line and culture conditions. However, the mechanisms underlying ammonia toxicity still are not entirely understood. It is suggested that ammonia accumulation disrupts electrochemical cell gradient and induces cytoplasm acidification which may lead to intracellular enzyme inactivity and apoptosis induction (Westlund and Häggström 1998). Additionally, there are reports that certain changes in N- and O- glycosylation of recombinant proteins in CHO cell culture are caused by high concentrations of exogenous ammonium (Borys et al. 1994; Andersen and Goochee 1995).

Since the consumption rates of glucose and glutamine as well as the accumulation rates of lactate and ammonia, reflect metabolic activities of cultured cells, monitoring of nutrient level and their potentially toxic waste-products has become a routine practice. Due to the need for efficient and reliable culture processes, understanding the consequences of increased levels of metabolic products in cell environment can be advantageous. Therefore in the present work, a CCO cell line was used to study the effects of various exogenous ammonium and lactate concentrations on cell viability. To our knowledge, there are no data about the sensitivity of fish cell lines to lactate and ammonia, so the results presented here may contribute to the change of this fact. In addition, we report on the influence of various glutamine concentrations on cell growth and metabolism, examining the lactate and ammonia production as well as the glucose and amino acid consumption. According to our earlier results, glutamine may be considered a major energy source for the CCO cell line since it was shown that the lack of glutamine in culture medium inhibits cell proliferation and the lack of glucose just decreases cell growth rate (Slivac et al. 2008). This confirms the standard concept of glucose and glutamine as substitutable and/or complementary nutrients in cellular catabolic and anabolic reactions, however indicating glutamine as a critical nutrient in this interrelation.

Materials and methods

Cell line and culture conditions

The channel catfish ovary cell line (CCO) was obtained from ATCC (CRL-2772). The cells were propagated in tissue culture flasks in DME medium (Sigma) supplemented with 5.56 mM glucose, 2 mM glutamine, 1 mM pyruvate and 5% fetal bovine serum (FBS, Gibco). The medium was buffered with $NaHCO_3$ and CO_2. The cell culture was maintained in an incubator at 30 °C in atmosphere consisted of humidified air (95%) and CO_2 (5%).

For the study of ammonia and lactate effect on cell growth, the cells were inoculated and left to attach in 24-well plates in the same medium formulation (500 μl per well) at initial cell density of about 5×10^4 cells ml^{-1}. After 24 h, medium in each well was exchanged with fresh one containing defined concentrations of ammonium-chloride or sodium-lactate (0, 1, 2.5, 5, 7.5, 10 and 20 mM).

For the investigation of influence of glutamine on cell growth and metabolism, cells were cultivated in

DME medium (Sigma) with or without glucose (5.56 mM), suplemented with four different glutamine concentrations (0, 1, 2 and 4 mM) during 6 days. The cells were cultivated in 24-well plates, and prior to this cultivation they had been propagated in tissue culture flasks in complete medium.

Trypan blue exclusion method was used for estimation of viable cells in Fuch-Rosenthal haemocytometer after detaching cells from the well surface with trypsin (0.25%, Gibco). Antibiotics were not used during the cultivation.

Analysis of metabolites

Glucose, lactate and ammonia in culture medium were quantified enzymatically. The glucose concentration was determined using Glucose-PAP colour-enzymatic assay kit (Herbos Sisak, Croatia). Lactate and ammonia were assayed by UV-test (Boehringer Mannheim/R-Biopharm, Darmstadt, Germany).

Amino acids were assayed by ion exchange liquid chromatography with an Amersham Biochrom 20 plus analyzer (Laboratory for Diagnosis of Metabolic Diseases, Clinical Hospital Center Zagreb, Zagreb, Croatia).

Metabolic parameter calculations

Specific rates of substrate consumption and metabolite production were calculated using the following formula:

$$Q_{Glc} = 2 \times 10^{-3} \left(C_{Glc,t0} - C_{Glc,t1} \right) / [(X_{t1} + X_{t0}) \times t];$$
$$Q_{Gln} = 2 \times 10^{-3} \left(C_{Gln,t0} - C_{Gln,t1} \right) / [(X_{t1} + X_{t0}) \times t];$$
$$Q_{Amm} = 2 \times 10^{-3} \left(C_{Amm,t0} - C_{Amm,t1} \right) / [(X_{t1} + X_{t0}) \times t];$$
$$Q_{Lac} = 2 \times 10^{-3} \left(C_{Lac,t0} - C_{Lac,t1} \right) / [(X_{t1} + X_{t0}) \times t];$$
$$Q_{AA} = 2 \times 10^{-3} \left(C_{AA,t0} - C_{AA,t1} \right) / [(X_{t1} + X_{t0}) \times t];$$

where Q is specific consumption rate (mmol cell^{-1} h^{-1}); C is the metabolite concentration (mmol cell^{-1} h^{-1}); t_0 and t_1 are the seeding and harvest times (d); X_{t1} and X_{t0} are the viable cell number at t_0 and t_1, cells ml^{-1}. Subscripts Glc, Gln, Lac, Amm and AA indicate glucose, glutamine, lactate, ammonia and amino acid, respectively.

The yield coefficients of lactate to glucose and ammonia to glutamine (mmol mmol^{-1}) were calculated from the formula:

$$Y_{Lac/Glc} = Q_{Lac}/Q_{Glc}$$
$$Y_{Amm/Gln} = Q_{Amm}/Q_{Gln}$$

Results and discussion

Influence of ammonium and lactate on CCO cell growth

In the first part of this work the effect of ammonium and lactate in a CCO cell line was studied. These metabolites are regularly produced in animal cell culture and are known to cause considerable decrease in cell biomass and product yield. Six different concentrations of ammonium chloride and sodium lactate were used and they were tested separately. The tested agents were added in 1 day old culture and the viable cell number was determined by trypan-blue exclusion method for the next 3 days (Fig. 1a).

The obtained results showed that the cell growth rate was reduced with the increase in ammonium as well as in lactate concentration (Table 1). Expectedly, the fastest cell growth was determined in culture medium without agents added. It was shown however that ammonium affects the cell growth more strongly than lactate. At relatively low concentrations of ammonium (2.5 mM) the growth rate was reduced by 44.2% while the same concentration of lactate reduced cell growth rate by 14%. Furthermore at a concentration of 5 mM, ammonium almost completely prevented the cell growth and even the reduction in cell number was observed during the last 2 days of cultivation. At cultures with 20 mM ammonium-chloride cell death was observed 24 h after treatment. The same effect did not occur in lactate treated cultures at any applied concentration. The significant inhibition effect of lactate started at concentration of 2.5 mM. Higher concentrations applied just intensified it by diminishing the final cell number and the growth rate (Fig. 1b). The glucose consumption rate did not change significantly with the addition of lactate, but it followed the decrease of growth rate in ammonium treated cultures (Table 1) indicating disturbed cell metabolism.

In order to investigate endogenous lactate production rate, determination of lactate concentration in culture medium of lactate treated cells was carried out (Fig. 1c). The results showed that the quantity of lactate in the control sample as well as in the media
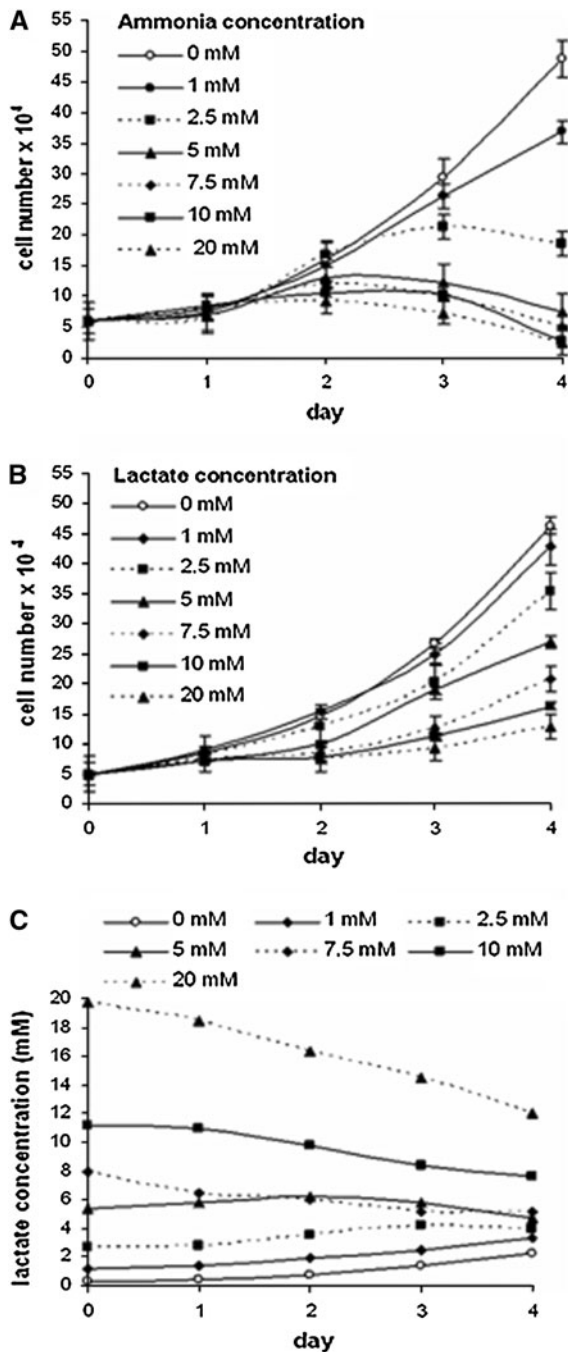
Fig. 1 Effects of exogenous ammonium and lactate in CCO cell culture. Cell growth in medium with added NH_4Cl (**a**) or sodium lactate (**b**). The concentrations applied were 0 (control) 1, 2.5, 5, 7.5, 10 and 20 mM. Lactate concentration in cell culture treated with sodium lactate (**c**)

sampled from cultures treated with 1 and 2.5 mM sodium lactate was steadily increasing during the whole cultivation period. Total lactate quantity was

considered to be the sum of initially added lactate and the lactate endogenously produced. The lactate production rate was rather similar in all three cultures, ranging between 8.3 and 9.2×10^{-11} mmol $cell^{-1}$ h^{-1}. However, in cell cultures with sodium lactate concentration of 7.5, 10 and 20 mM, the lactate level was found to decrease during the whole cultivation period. Exception was the culture at 5 mM sodium lactate where the lactate level was raising for the first 2 days and reached 6.2 mM at rate (Q_{Lac}) 9.7 $\times 10^{-11}$ mmol h^{-1} $cell^{-1}$ (Table 1). On day 3 the trend changed and the lactate concentration started to drop at rate -13×10^{-11} mmol h^{-1} $cell^{-1}$ finally reaching 4.8 mM. The phenomenon of lactate level decrease may be related to the activity of lactate dehydrogenase in the culture medium. The metabolic role of this intracellular enzyme is conversion of lactate into pyruvate, but it was probably released into the medium from damaged or dead cells since the small quantity of detached (i.e. damaged) cells was regularly present in treated cultures due to the stressful pH value decrease caused by addition of sodium lactate. Alternative explanation could be that the high lactate concentrations stimulated cells to initiate lactate consumption. There are reports about the lactate utilization by CHO cells, but this is suggested to happen in non-oxidative manner in specific nutritive conditions when galactose is present in medium and glucose is scarce (Altamirano et al. 2006). However, in our research glucose was always sufficient and was consumed with similar rate in all lactate treated cultures.

The comparison of influence of lactate and ammonium on cell growth rates showed that a similar inhibitory effect occurs when lactate concentration is 2–3 times higher than that of ammonium. This is in agreement with several authors who report about the ammonium and lactate toxicity in a variety of mammalian cell lines (Cruz et al. 2000; Mirabet et al. 1997; Ozturk et al. 1992). According to them, BHK 21, Jurkat cells and hybridoma cells are almost 10 times more sensitive to ammonium than to lactate, which is considerably higher compared to our results on CCO cell line. Besides affecting the cell growth, ammonium chloride also induced morphological changes in treated CCO cells. In cells that were attached to the plate cytosolic vacuolization was observed (Fig. 2b) compared to control cells (Fig. 2a). This change appeared in all treated cultures

Table 1 Specific growth rate (μ) and specific glucose consumption rate (Q_{Glc}) in CCO cultures treated with different concentrations of ammonium-chloride or sodium lactate. Specific lactate production rate (Q_{Lac}) was determined for lactate treated cells only

Ammonium/Lactate concentration (mM)	Ammonium treated cells		Lactate treated cells			
	μ (h^{-1})	Q_{Glc} (10^{-11} mmol h^{-1} cell^{-1})	μ (h^{-1})	Q_{Glc} (10^{-11} mmol h^{-1} cell^{-1})	Q_{Lac} (10^{-11} mmol h^{-1} cell^{-1})	
Control	0.0260	6.6	0.0235	7.2	8.3	
1 mM	0.0212	6.2	0.0218	6.9	9.2	
2.5 mM	0.0143	5.9	0.0201	7.2	8.8	
5 mM	0.0116	1.6	0.0186	7.8	9.7*	−13.8**
7.5 mM	0.00464	0.7	0.0139	7.3	−11.5	
10 mM	0.0042	0.4	0.0112	7.8	−35.4	
20 mM	–	–	0.0074	6.3	−85.7	

*Value for the first 2 days of culture; **Value beyond day 3 of culture
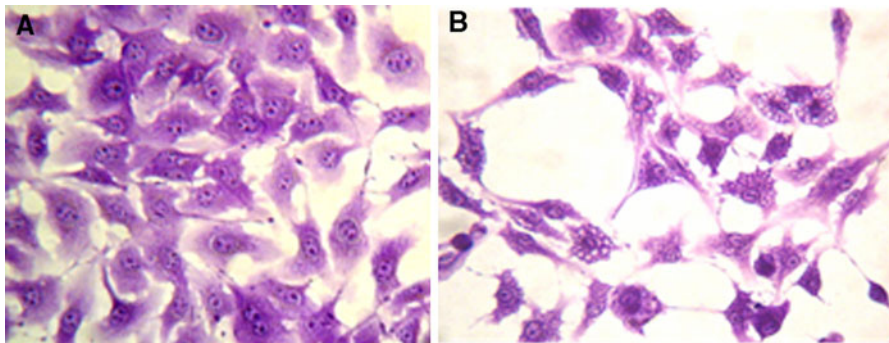
Fig. 2 Morphology of CCO cells: control (**a**) and cells treated with 10 mM NH$_4$Cl (**b**). Widespread cytosol vacuolization can be observed

and the percentage of affected cells rose with the increase of ammonium chloride concentration and the time of exposure. This is well known effect which occurs in many cell cultures because the ammonia and other weak bases accumulate within the cell lysosomes and markedly change the pH of intracellular compartments. Apoptotic cell death triggered by such structural and physiological alterations is often recognized as the main reason for reduction of cell number yield in ammonia containing culture medium.

Influence of glutamine on CCO cell growth

In the second part of this work we studied growth and metabolism of CCO cells cultivated in glucose-free and glucose-supplemented medium at various concentrations of glutamine. Our earlier investigation of CCO cell growth characteristics led us to the conclusion that glutamine may play a more important role in cell culture propagation than glucose, since the deficiency of glutamine inhibited cell proliferation while the lack of glucose just retarded cell growth affecting the uptake rate of nutrients (Slivac et al. 2008). Thus to clarify the significance of glutamine in CCO cell metabolism, four different glutamine concentrations were prepared (0, 1, 2 and 4 mM) in culture medium with 5.56 mM glucose. In medium without glucose, glutamine concentrations were 1, 2 and 4 mM avoiding conditions without both nutrients. Cells were cultivated in these media until the fastest growing culture reached stationary growth phase (Fig. 3a).

The results showed that increased glutamine concentration in complete medium slightly accelerated the cell growth rate (μ) as well as the glutamine consumption rate (Q_{Gln}) by a factor of 1.2 and $\sim$1.5 respectively (Table 2). Thus the highest final cell number was reached in medium with 4 mM glutamine (160.2×10^4 cells) and the lowest cell number in

Fig. 3 CCO cell growth
(**a**), glucose consumption
(**b**), ammonia accumulation
(**c**) and lactate accumulation
(**d**) in glutamine-free
medium and in complete
and glucose-free medium
with different glutamine
concentrations
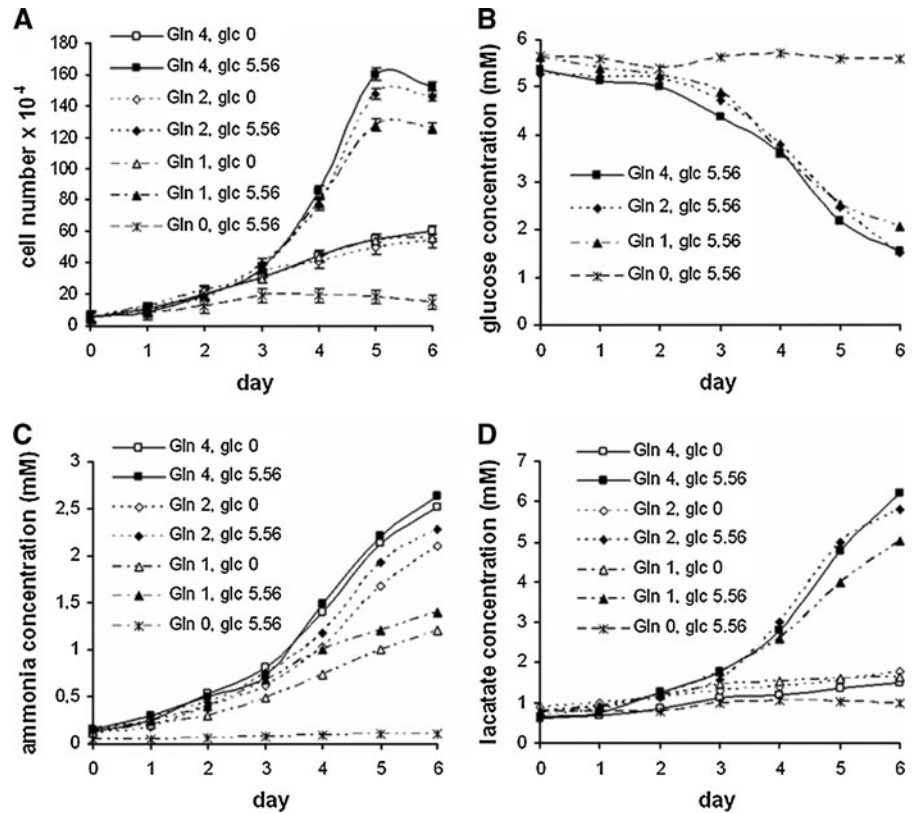
culture with 1 mM glutamine (128×10^4 cells).
However, the retardation of cell growth in the latter
condition, which was most evident on the fifth day of
cultivation, might have happened due to glutamine
depletion determined by amino acid analysis. It seems
that in this case glutamine concentration was the
limiting factor, since in cultures with higher glutamine
levels (2 and 4 mM) there was enough residual
glutamine left (0.54 and 2.4 mM) until the end of
the cultivation. The relatively low cell number yield in
culture without glutamine indicates that glutamine
plays critical role in CCO cell proliferation. The
observed cell number increase, of barely two genera-
tions, was achieved in the first 3 days of cultivation,
thus suggesting that the cell growth might have been
driven by intracellular glutamine supplies originating
from cell propagation in complete medium. Glucose,
when present in culture medium, was always con-
sumed faster than glutamine, however the value of
glucose consumption rate (Q_{Glc}) decreased with the
increase of initial glutamine concentration. The
exception was cell culture without glutamine where
glucose was barely consumed (Fig. 3b). This trend is

in agreement with many reports on cell line nutrient
utilization and confirms supposed complementary and
reciprocal regulation of glucose and glutamine (Cruz
et al. 1999a).

In glucose-free medium the final cell number and
specific growth rate did not change significantly with
the increase in glutamine concentration ranging
between 0.0191 h^{-1} and 0.0199 h^{-1} at 54.6×10^4
and 59.9×10^4 cells, respectively. The glutamine
uptake rate was again dependent on initial glutamine
concentration, increasing with the glutamine concen-
tration increase. Its value was considerably higher
than Q_{Gln} determined in complete medium probably
because cells had to compensate for the glucose
depletion and to shift to glutaminolysis as introduc-
tion to energetically abundant TCA cycle. Similar
explanation was given by Barnabé and Butler (2000)
after research on nutrient uptake of murine hybrid-
oma cells and Ehrlich ascites tumor cells. Ljunggren
and Häggström (1994) report about glutamine enter-
ing TCA cycle through glutamate dehydrogenase
pathway after experiments with hybridoma cell
cultivation in glucose limited conditions.

Table 2 Specific cell growth rate (μ), specific glucose and glutamine consumption rates (Q_{Glc}, Q_{Gln}), specific lactate and ammonia production rates (Q_{Lac}, Q_{Amm}) and yield coefficients of lactate to glucose and ammonia to glutamine ($Y_{Lac/Glc}$, $Y_{Amm/Gln}$) were determined in seven CCO cell cultures with different medium compositions. Cells were cultivated in glutamine-free medium and in glucose free-medium and complete medium with three different glutamine concentrations

Medium	μ (h^{-1})	Q_{Glc} (10^{-11} mmol cell^{-1} h^{-1})	Q_{Lac} (10^{-11} mmol cell^{-1} h^{-1})	$Y_{Lac/Glc}$ (mmol mmol^{-1})	Q_{Gln} (10^{-11} mmol cell^{-1} h^{-1})	Q_{Amm} (10^{-11} mmol cell^{-1} h^{-1})	$Y_{Amm/Gln}$ (mmol mmol^{-1})
Gln 4 mM, glc 0 mM	0.0199	–	2.61	–	3.86	6.88	1.78
Gln 4 mM, glc 5.56 mM	0.0308	3.10	3.94	1.27.	2.72	3.25	1.20
Gln 2 mM, glc 0 mM	0.0191	–	2.52	–	3.13	5.82	1.86
Gln 2 mM, glc 5.56 mM	0.0267	4.12	4.80	1.16	1.58	2.16	1.36
Gln 1 mM, glc 0 mM	0.0198	–	2.7	–	1.85	3.04	1.64
Gln 1 mM, glc 5.56 mM	0.0229	5.28	5.64	1.07	1.11	1.45	1.31
Gln 0 mM, glc 5.56 mM	0.0148	1.18	0.31	0.26	–	0.47	–

Ammonia production was expectedly most abundant in cultures with highest level of glutamine due to cell metabolic activity or spontaneous glutamine degradation. Cells in glucose free medium produced ammonia at rate (Q_{Amm}) markedly higher than cells in complete medium which reflects already mentioned importance of glutamynolysis and transamination pathway in the glutamine metabolism. The glutamine to ammonia yield ($Y_{Amm/Gln}$) ranged between 1.2 and 1.86 mol $\times$ mol^{-1} and was slightly increased in glucose free medium indicating that there was need to use more glutamine as an energy source. The values obtained are in agreement with reports on mammalian and fish cell lines (Cruz et al. 1999b; Linz et al. 1997; Chen et al. 2005). It was to be noted that effects of ammonia accumulation might have been evident in cultures with initial 2 and 4 mM glutamine, regardless of glucose presence in the medium. In both conditions the final ammonia concentration reached over 2 mM and 2.5 mM, respectively (Fig. 3c). This may have further affected cell proliferation since, according to our previous results, cells treated with 2.5 mM NH$_4$Cl displayed an almost twofold decrease in growth rate compared to untreated cells.

Lactate accumulation was typically dependant on glucose presence in culture medium. In media with glucose, lactate concentration reached between 5 and 6.2 mM (Fig. 3d). However the lactate production rate (Q_{Lac}) was also affected by glutamine since the fastest lactate production was observed in cultures with the lowest initial glutamine concentration. At the same time the lactate to glucose yield ($Y_{Lac/Glc}$) increased with the increase of glutamine level. The same effect was not observed in glucose free media. There was less lactate produced and all of it at similar and relatively low production rate. It is well known that in culture conditions without glucose, lactate can be derived from pyruvate generated from small nonessential amino acids like serine, alanine, glycine and cysteine as well as from glutamine via TCA cycle intermediates.

Although glutamine is the most relevant amino acid in cell culture, it is also important to consider metabolism of other amino acids in culture medium. The main purpose of amino acids is their integration into peptides during protein synthesis, however they also have a considerable role in anabolic and catabolic reactions when either glutamine or glucose

Table 3 Specific consumption rates of amino acids (Q_{AA}) (excluding glutamine) in CCO cell culture. Amino acids were analyzed in glutamine-free and glucose-free medium, as well as in complete media with different concentrations of glutamine

Q_{AA} (10^{-11} mmol cell^{-1} h^{-1})	Culture medium composition				
	Gln 2 mM, glc 0 mM	Gln 0 mM, glc 5.56 mM	Gln 1 mM, glc 5.56 mM	Gln 2 mM, glc 5.56 mM	Gln 4 mM, glc 5.56 mM
Q_{Ser}	0.90	1.27	0.45	0.31	0.26
Q_{Gly}	−1.62	−3.01	−0.40	−0.45	−0.48
Q_{His}	−0.05	0.06	0.03	0.04	0.05
Q_{Thr}	−0.15	2.6	0.91	0.70	0.58
Q_{Ala}	−0.44	−1.35	−0.96	−0.76	−0.71
Q_{Arg}	1.08	2.59	0.05	0.36	0.48
Q_{Tyr}	0.16	0.16	0.02	0.036	0.04
Q_{Val}	0.40	0.02	0.38	0.30	0.22
Q_{Met}	0.14	0.08	0.23	0.10	0.06
Q_{Cys2}	0.33	0.45	0.20	0.14	0.11
Q_{Ile}	0.72	0.91	0.45	0.34	0.20
Q_{Leu}	0.74	0.72	0.54	0.36	0.14
Q_{Phe}	−0.09	−0.33	0.04	0.01	0.01
Q_{Lys}	0.24	0.89	0.47	0.15	0.06
Q_{Glu}	−0.79	0	−0.03	−0.15	−0.28
Q_{Asn}	−0.17	−0.13	0	−0.05	−0.02
Q_{Asp}	0.06	−0.92	−0.01	−0.02	−0.03

concentration are not sufficient. Table 3 presents amino acid consumption rate values (Q_{AA}) of CCO cells cultivated in glucose-free medium and in glutamine-free and complete media with three different glutamine concentrations (1, 2 and 4 mM). The majority of the analyzed amino acids were regularly consumed (Ser, Lys, Leu, Ile, Cys and Arg) while others were produced and released into the medium (Ala, Gly, Asn and Asp). There were also amino acids that were either consumed or produced, depending on glucose and glutamine presence (Thr, Phe). Overall amino acid consumption was markedly increased when glucose or glutamine were absent in order to compensate for missing nutrients. This explains why Ser, Lys, Leu, Ile, Arg, Met and Cys consumptions in complete medium decreased with the increase in glutamine concentration. The arrest of threonine consumption in medium without glucose remained unclear since this amino acid could be converted into pyruvate or succinyl-CoA and enter the TCA cycle. Both, Gly and Ala were abundantly produced by CCO cells. Glycine often derives from serine metabolism and therefore its production rate is related to serine utilization. Alanine is considered as

overflow metabolite and was produced at significant rate when glucose was present in the medium. It derived from glutamic acid and pyruvate which means that in complete media its production could increase; in medium without glucose alanine production was consequently reduced almost by twofold. The production of alanine was enhanced in medium with a decrease in glutamine concentration which means that there was eventually deficit of glutamate and thereby pyruvate was destined for conversion into lactate or acetyl-CoA. These observations are in agreement with Mancuso et al. (1998) and Cruz et al. (1999b) who investigated amino acid utilization in hybridomas and BHK cells, respectively. Chen et al. (2005) found that in glutamine and glucose free culture of another fish cell line (CHSE) pyruvate was derived from amino acids and covered energy deficiency.

Conclusion

Determination of CCO cell growth and metabolic characteristics, performed in our study, can be

beneficial for extension of the applications with this cell line. Our results showed that both extracellular ammonium and lactate reduced cell growth rate, yet in different range and mode. Ammonium was more severe in generating inhibitory effects than lactate because three times lower concentrations of ammonium-chloride were required to achieve the cell growth retardation similar to that induced by sodium lactate. Furthermore, at relatively high ammonium concentrations (10 and 20 mM) growth arrest occurred together with morphological changes of the cytosol while in cultures treated with the same concentrations of lactate, progressive growth rate decrease was observed followed by a considerable cell detachment.

The investigation of the glutamine effect on CCO cell growth showed that nutrient requirements of this cell line mainly depended on glutamine rather than glucose. Glutamine stimulated cell growth and reduced glucose utilization whereas its deficiency in culture medium induced cell growth arrest. Glucose also had a significant role in CCO cell proliferation since its deficiency reduced cell growth rate. However, in glucose deficient culture medium cells were still metabolically active using glutamine and other amino acids in order to meet their catabolic and anabolic needs. Results obtained are useful for design of an optimal nutrition medium and improvement of ongoing CCO cell application.

References

Altamirano C, Illanes A, Becerra S, Cairó JJ, Gòdia F (2006) Considerations on the lactate consumption by CHO cells in the presence of galactose. J Biotechnol 125:547–556

Andersen DC, Goochee CF (1995) The effect of ammonia on the O-linked glycosylation of granulocyte colony-stimulated factor produced by Chinese hamster ovary cells. Biotechnol Bioeng 47:96–105

Barnabé N, Butler M (2000) The effect of glucose and glutamine on the intracellular nucleotide pool and oxygen uptake rate of a murine hybridoma. Cytotechnology 3:47–57

Borys MC, Linzer DIH, Papoutsakis ET (1994) Ammonia affects the glycosylation patterns of recombinant mouse placental lactogen-I by Chinese hamster ovary cells in a pH dependent manner. Biotechnol Bioeng 43:505–514

Bowser PR, Plumb JA (1980a) Fish cell lines: establishment of a line from ovaries of channel catfish. In Vitro 16:365–368

Bowser PR, Plumb JA (1980b) Channel catfish virus: comparative replication and sensitivity of cell lines from channel catfish ovary and the brown bullhead. J Wildl Dis 16:451–454

Chen J, Sun X, Zhang Y (2005) Growth and metabolism of marine fish Chinook salmon embryo cells: response to lack of glucose and glutamine. Biotechnol Lett 27:395–401

Cruz HJ, Ferreira AS, Freitas CM, Moreira JL, Carrondo MJT (1999a) Metabolic responses to different glucose and glutamine levels in baby hamster kidney cell cultures. Appl Microbiol Biotechnol 51:579–585

Cruz HJ, Moreira JL, Carrondo MJT (1999b) Metabolic shifts by nutrient manipulation in continuous cultures of BHK cells. Biotechnol Bioeng 66:105–113

Cruz HJ, Freitas CM, Alves PM, Moreira JL, Carrondo MJT (2000) Effects of ammonia and lactate on growth, metabolism and productivity of BHK cells. Enzyme Microb Technol 27:43–52

Glacken MW, Adema E, Sinskey AJ (1988) Mathematical descriptions of hybridoma culture kinetics: I. Initial metabolic rates. Biotechnol Bioeng 32:491–506

Linz M, Zeng AP, Wagner R, Deckwer WD (1997) Stoichiometry, kinetics, and regulation of glucose and amino acid metabolism of a recombinant BHK cell line in batch and continuous cultures. Biotechnol Prog 13:453–463

Ljunggren J, Häggström L (1994) Catabolic control of hybridoma cells by glucose and glutamine limited fed batch cultures. Biotechnol Bioeng 44:808–818

Mancuso A, Sharfstein ST, Fernandez EJ, Clark DS, Blanch HW (1998) Effect of extracellular glutamine concentration on primary and secondary metabolism of a murine hybridoma: an in vivo 13 nuclear magnetic resonance study. Biotechnol Bioeng 57:172–186

Mirabet M, Navarro A, Lopez A, Canela E, Mallol J, Lluis C, Franco R (1997) Ammonium toxicity in different cell lines. Biotechnol Bioeng 56:530–537

Newland M, Kamal MN, Greenfield PF, Nielsen LK (1994) Ammonia inhibition of hybridomas propagated in batch, fed-batch, and continuous culture. Biotechnol Bioeng 43:434–438

Omasa T, Higashiyama K, Shioya S, Suga K (1992) Effects of lactate concentration on hybridoma culture in lactate-controlled fed-batch operation. Biotechnol Bioeng 39:556–564

Ozturk SS, Riley MR, Palsson BO (1992) Effects of ammonia and lactate on hybridoma growth, metabolism, and antibody production. Biotechnol Bioeng 39:418–431

Patel SD, Papoutsakis ET, Winter JN, Miller WM (2000) The lactate issue revisited: novel feeding protocols to examine inhibition of cell proliferation and glucose metabolism in hemotopoietic cell cultures. Biotechnol Prog 16:885–892

Slivac I, Gaurina Srček V, Radošević K, Porobić I, Bilić K, Fumić K, Kniewald Z (2008) Growth characteristics of channel catfish ovary cells–influence of glucose and glutamine. Cytotechnology 57:273–278

Stingley RL, Gray WL (2000) Transcriptional regulation of the channel catfish virus genome direct repeat region. J Gen Virol 81:2005–2010

Tan F, Wang M, Wang W, Lu Y (2008) Comparative evaluation of the cytotoxicity sensitivity of six fish cell lines to four metals in vitro. Toxicol In Vitro 22:164–170

Westlund A, Häggström L (1998) Ammonium ion transport by the $Na^+K^+2Cl^-$-cotransporter induces appoptosis in hybridoma cells. Biotechnol Lett 20:87–90