Dependence on Glucose Limitation of the $p$CO$_2$ Influences on CHO Cell Growth, Metabolism and IgG Production

Shinya Takuma,1,3 Chikashi Hirashima,3 James M. Piret1,2

1Michael Smith Laboratories, University of British Columbia, 2185 East Mall, Vancouver, British Columbia, V6T 1Z4, Canada; telephone: 604-822-5835; fax: 604-822-2114; e-mail: jpiret@chml.ubc.ca
2Department of Chemical & Biological Engineering, University of British Columbia, 2185 East Mall, Vancouver, British Columbia, V6T 1Z4, Canada
3Bio-product Technology Research Department, Chugai Pharmaceutical Co., Ltd., 5-1, 5-Chome Ukima, Kita-ku, Tokyo, 115-8543, Japan

Received 8 October 2005; accepted 30 January 2007
Published online 22 February 2007 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/bit.21376

ABSTRACT: The culture levels of glucose and CO$_2$ have been reported to independently have important influences on mammalian cell processes. In this work the combined effects of glucose limitation and CO$_2$ partial pressure ($p$CO$_2$) on monoclonal antibody (IgG) producing Chinese Hamster Ovary cells were investigated in a perfusion reactor operated with controlled cell specific medium feed rate, pH and osmolality. Under high glucose conditions (14.3 ± 0.8 mM), the apparent growth rate decreased (from 0.021 to 0.009 h$^{-1}$) as the $p$CO$_2$ increased to 220 mmHg, while the cell specific IgG productivity was almost unchanged. The lactate yield from glucose was not affected by $p$CO$_2$ up to 220 mmHg and glucose was mainly converted to lactate. A feed medium modification from high (33 mM) to low (6 mM) glucose resulted in <0.1 mM glucose in the culture. As a result of apparently shifting metabolism towards the conversion of pyruvate to CO$_2$, both the ratio of lactate to glucose and the alanine production rate were lowered (1.51–1.14 and 17.7–0.56 nmol/10$^6$ cells h, respectively). Interestingly, when the $p$CO$_2$ was increased to 140 mmHg, limiting glucose resulted in 1.7-fold higher growth rates, compared to high glucose conditions. However, at 220 mmHg $p$CO$_2$ this beneficial effect of glucose limitation on these CHO cells was lost as the growth rate dropped dramatically to 0.008 h$^{-1}$ and the IgG productivity was lowered by 15% ($P < 0.01$) relative to the high glucose condition. The IgG galactosylation increased under glucose-limited compared to high-glucose conditions

© 2007 Wiley Periodicals, Inc.

KEYWORDS: carbon dioxide; glucose; CHO cell physiology; perfusion culture; monoclonal antibody production; IgG galactosylation

Introduction

Transfected mammalian cells are powerful tools for producing proteins of interest, especially those that need complex post-translational modification, such as glycosylation. Much effort has been expended to increase culture process yields and productivity. Recently, a number of monoclonal antibodies have been approved for therapeutic application and many more are in clinical trials. The commonly used mammalian cell lines are Chinese Hamster Ovary (CHO) cells, and the two murine myeloma lines SP2/0 and NS0 (Chu and Robinson, 2001). Because of the high doses required for many antibody therapeutics compared to other products, the high volumetric productivity of perfusion culture could make it a particularly useful process for monoclonal antibody production. In fact, a substantial number of cell culture products approved by the Food and Drug Administration (FDA), are produced using perfusion cultures (Chu and Robinson, 2001).

There can be significant CO$_2$ accumulation in culture, especially at larger scales or at high cell density (Schmelzer and Miller, 2002). Indeed, a $p$CO$_2$ level of 171 mmHg was reported for a 500 L CHO cell culture at 11 × 10$^6$ cells/mL (Gray et al., 1996), and 179 mmHg for 1000 L CHO cell fed-batch cultures (Mostafa and Gu, 2003). CO$_2$ can influence not only cell growth and protein productivity, but also product quality. Polysialylation levels of the neural cell adhesion molecule (NCAM) from CHO cells decreased with increasing $p$CO$_2$ (Zanghi et al., 1999). Also, the monosaccharide content of IgG$_{2a}$ produced by a
hybridoma changed depending on the pCO2 level (Schmelzer and Miller, 2002), however there was no change in the glycosylation of tissue plasminogen activator (t-PA) between 36 and 250 mmHg pCO2 (Kimura and Miller, 1997). The difficulty of reducing bioreactor pCO2 levels to the physiological range (31–54 mmHg, Altman and Dittmer, 1971) has motivated a search for pCO2 ranges under which the detrimental effects are negligible or acceptable (deZengotita et al., 2002; Schmelzer and Miller, 2002). Recently, a novel approach to decrease pCO2 levels was reported by Matanguihan et al. (2001).

The complex nature of CO2 dissolution in medium is described in detail by deZengotita et al. (2002). In brief, dissolved CO2 is hydrated to produce carbonic acid and, in the range of pH for mammalian cell culture (around 7.0), the equilibration equation can be simplified to:

\[ \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{HCO}_3^- \quad (1) \]

where the pK_a = 6.02 at 37°C. If the culture pH is not controlled, accumulation of CO2 will lead to a reduction of culture pH. If the pH is controlled by base addition, the osmolality of the culture increases, due to the cation of the added base, shifting the equilibrium towards HCO3-. There may be detrimental effects on the cell survival, growth or productivity if either the pH or the osmolality shift outside their optimal ranges (Ozturk and Palsson, 1991b; Zhu et al., 2005). In some cases, however, protein production can be increased at higher osmolality (Lee and Lee, 2000). In addition to these indirect effects of CO2, elevated levels have been reported to reduce intracellular pH by ~0.2 units (deZengotita et al., 2002).

Lactate accumulation can also inhibit cell growth and/or protein productivity, especially indirectly by increasing the need for pH control in bioreactors (Lao and Toth, 1997). Lactate accumulation has been reduced most often by limiting glucose levels (Europa et al., 2000; Glacken et al., 1986; Zhou et al., 1997). The use of alternative sugars, such as galactose (Altamirano et al., 2001b), can also reduce lactate production.

Many investigations of culture processes are limited to analyzing one parameter at a time. In particular, though the above reports examined the effects of low glucose concentration or high pCO2, none reported the interaction between these two conditions. Since limiting glucose in the culture provides benefits from lowering the lactate concentration and high CO2 concentration can be a problem when cultures are scaled up, in this work we examined the combined effects of low glucose concentration and high pCO2 on CHO cell physiology and IgG production. In order to decouple the pCO2 effects from pH and osmotic changes, we used a perfusion culture operated with controlled pCO2, pH and osmolality, while changing the glucose composition of the feed medium.

**Materials and Methods**

**Cell Line and Subcultures**

A CHO cell line producing recombinant immunoglobulin G (IgG) was created by transfecting the dihydrofolate reductase (DHFR) negative CHO DXB-11 cell line with a recombinant vector containing both a DHFR and a humanized antibody gene. The transfected genes were amplified by the DHFR inhibitor, methotrexate (MTX), and a high IgG producer was selected and cloned. The cells were grown in a proprietary serum-free medium containing an Insulin like Growth Factor-1 (IGF-1) analog (Morris and Schmid, 2000) as the only protein and with 500 nM MTX. This cell line is stable in culture for over 100 days in the presence of 500 nM MTX. Seed cells were prepared in 100–1000 mL spinner flasks by subculturing every 3 days from an initial cell density of 1.0 x 10^5 cells/mL, and incubated in 5% CO2 at 37°C and 80 rpm.

**Batch Culture**

Batch culture experiments were conducted using MTX-free serum-free medium, with NaOH and NaCl added so as to adjust the initial pH and osmolality to 6.8 and 290 mOsm/kg, respectively, at the different pCO2. The bioreactor (ABLE, Japan) had a 1 L working volume. A mixed gas of air, O2 and CO2 was introduced through Teflon® tubing immersed in the culture liquid in order to maintain the dissolved oxygen (pO2) at 50% air saturation and the CO2 in the gas phase at the specified concentrations. The head pressure was approximately 0.1 MPa, the temperature at 37°C and the agitation at 80 rpm. The bioreactor was inoculated at 1–2 x 10^5 cells/mL, with the split ratio of one tenth from the subculture.

**Perfusion Culture**

The serum-free perfusion Medium 1 (M1) and Medium 2 (M2) contained 33 and 6 mM glucose, respectively, and 500 nM MTX. The concentrations of NaOH and NaCl were adjusted to keep the bioreactor pH (6.9–7.0) and osmolality (300–320 mOsm/kg) within narrow ranges, i.e. with increasing pCO2 more NaOH and less NaCl were added to the feed medium. The perfusion reactor consisted of a 1 L fermentor (600 mL working volume, LH Fermentation, UK), and an ultrasonic cell retention device (BioSep ADI 1015, Applikon, CA). The BioSep was operated at a power input of 3 W, duty cycle run time of 45 s, with a stop time of 9 s, harvest flow rate of 2 mL/min, air-backflush frequency of 4 h^-1 (Gorenflo et al., 2003) using programmed controllable peristaltic pumps (Master Flex, Cole-Parmer, IL). Air, O2
and CO₂ were used to maintain the pO₂ at 50% air saturation and the pCO₂ at the specified levels. The O₂ and CO₂ were added, along with air, through Teflon® tubing immersed in the culture liquid, while air was also delivered through the headspace. The pCO₂ value measured with an off-line analyzer (Stat Profile 10 Plus, Nova Biomedical, MA) was used to control culture pCO₂. Moreover, a pCO₂ probe (YSI 8500, YSI, Yellow Springs, OH) was used to monitor the pCO₂ (Pattison et al., 2000). The head pressure was 0.1 MPa, the temperature 37°C and the agitation 120 rpm. The fermentor was inoculated at (1−2) × 10⁵ cells/mL, and in the perfusion phase the cell concentration was maintained at 1.0 × 10⁷ cells/mL by cell bleeding. Fresh medium was fed (once an hour) at a constant average feed rate per cell (165 μL/10⁶ cells day) by predicting the cell density until the next sampling time, based on the last cell density and the growth rate. After each culture condition change, the process reached a pseudo-steady state, where seven consecutive samples were taken. All pseudo-steady states satisfied the criteria that the pCO₂ and lactate concentration coefficients of variance (CV) decreased to less than 0.1. Pseudo-steady state durations were 3.0−7.1 days, depending on the sampling frequency. Overall, the perfusion culture results described were replicated one or two times by two additional cultures, with only one qualitative inconsistency that is described.

Analytical Methods

Cell density and viability were estimated using a hemocytometer and the trypan blue dye-exclusion method. For the analysis of glucose and lactate concentrations in the batch culture, a YSI 2700 SELECT analyzer (YSI, Yellow Springs, OH) was used. For the perfusion cultures, medium pH, pCO₂, pO₂, glucose (above 4.4 mM), lactate and ammonium concentrations were determined off-line using an automated analyzer (Stat Profile 10 Plus, Nova Biomedical, MA). The glucose concentrations below 4.4 mM were determined using a glucose assay kit (Diagnostic Chemicals, PEI). The actual efficiency was approximately 98%, on average, but it was not practical to measure the separation efficiency at every sample. At the average separation efficiency of 98%, then the maximum errors of μ and kₐ estimation were calculated to be 0.001 h⁻¹ (CV = 15%) and 0.0002 h⁻¹ (CV = 11%), respectively for any pseudo-steady state. The effects of the hourly bleed rate and the sampling volume were taken into account when calculating μ and kₐ. Because a rapid bleed flow rate was used (80 mL/min, for ~15 s), it was assumed that there was negligible settling of the cells during the bleed. However, since 3 mL of suspended cells remained in the vertical outflow tube after each hourly bleed, it was assumed that these cells settled back into the bioreactor.

For the analysis of IgG glycosylation (Kondo et al., 1990), cultured medium was sampled at the end of each pseudo-steady state since, by then, the medium was diluted by more than 3 culture volumes during the pseudo-steady state. The IgG was purified from the centrifuged supernatant of the culture medium using a 2 mL Econo-Pac Protein A column (BIORAD, CA).

The oligosaccharide of the purified IgG was released using an N-Glycosidase F (Roche Diagnostics, Switzerland) at 37°C for 18 h and labeled with 2-aminopyridine at the reducing terminus using the PALSTATION Pyridylation Reagent Kit (TaKaRa, Japan). This labeled oligosaccharide was analyzed by reverse-phase chromatography. Each peak was assigned by both mass spectrometry and standards for the oligosaccharides. The column used was Shim-Pack CLC-ODS(M), 250 mm × 4.6 mm ID, 10 nm pore size (SHIMADZU, Japan), and a Waters 474 fluorescence detector set at excitation 320 nm and emission 400 nm. Buffer A was 10 mM phosphate buffer (pH 3.8) and buffer B was buffer A plus 0.5% (v/v) 1-butanol. The gradient employed was 20%−50% buffer B over 60 min, 50%−80% buffer B over 5 min, 80% buffer B for 5 min, and re-equilibration in starting conditions for 30 min. The flow rate was 1.0 mL/min.

Estimation of Specific Rates

Cell specific growth (μ) and death (kₐ) rates were obtained from mass balances of the total and the nonviable cells (deZengotita et al., 1998):

\[
\frac{dX_t}{dt} = \mu X_v \tag{2}
\]

\[
\frac{dX_{nv}}{dt} = k_d X_v \tag{3}
\]

where \(X_t\), \(X_v\), and \(X_{nv}\) were the total, viable and nonviable cell concentrations, respectively. These equations were integrated to calculate μ and kₐ assuming that the efficiency of the ultrasonic cell retention device was 100% (given the relatively slow harvesting compared to maximum capacity). The actual efficiency was approximately 98%, on average, but it was not practical to measure the separation efficiency at every sample. At the average separation efficiency of 98%, then the maximum errors of μ and kₐ estimation were calculated to be 0.001 h⁻¹ (CV = 15%) and 0.0002 h⁻¹ (CV = 11%), respectively for any pseudo-steady state. The effects of the hourly bleed rate and the sampling volume were taken into account when calculating μ and kₐ. Because a rapid bleed flow rate was used (80 mL/min, for ~15 s), it was assumed that there was negligible settling of the cells during the bleed. However, since 3 mL of suspended cells remained in the vertical outflow tube after each hourly bleed, it was assumed that these cells settled back into the bioreactor.

The cell specific glucose uptake rate (CSGUR), and production rates of lactate (CSLPR), ammonium (CSAPR) and IgG (CSIgGPR) were calculated from analysis of sequential samples, the feed medium and the feed rate, then divided by the integral of viable cells during the time interval. Amino acid consumption and production
rates were calculated in a similar fashion. The feed medium and four samples out of each pseudo-steady state (normally every other one) were analyzed for the 19 amino acid concentrations. This provided three cell specific consumption or production rates for each amino acid in each pseudo-steady state.

Statistical Analysis

The statistical significance of differences between pseudo-steady states was assessed by a two-tailed $t$ test. To investigate two parameter correlations, regression analysis was performed using a linear model. A linear model of the galactosylation response to glucose and $p\text{CO}_2$ (without interactions) and ANOVA were used to test the significance of the two variables.

Results and Discussion

Batch Culture

The effects of CO$_2$ were first screened in batch culture using CO$_2$ gas inlet controlled at 5, 20 or 40% (i.e. no direct $p\text{CO}_2$ control). The pH was kept constant at 6.8 by adding a solution of 1 M Na$_2$CO$_3$. The osmolality, however, increased by $\sim$100 mOsm/kg, mainly during the second half of the culture (Fig. 1a). Since the batch culture osmolality was

![Graphs](image)

**Figure 1.** Time profiles of CHO cell batch culture at 5% (○), 20% (□) and 40% (△) CO$_2$ gas inlet concentrations. (a) Osmolality changes. (b) Viable cell density. (c) IgG concentration.
below 350 mOsm/kg until day 6, a preliminary assessment of the effects of CO₂ could be drawn from this growth phase period. The cell growth was highly inhibited with 40% CO₂ (Fig. 1b). The final IgG concentration at day 10 was almost the same for 5 and 20% CO₂ but was much less for 40% CO₂ (Fig. 1c). The CSIgGPR, CSGUR and CSLPR were similar for 5 and 20% CO₂, but had lower values at 40% CO₂ (Table I). Since the cell growth at 40% CO₂ was so low, a pCO₂ of 220 mmHg (the value corresponding to 30% CO₂ at 0.1 MPa), was chosen as the maximum for the remainder of the experiments, where pCO₂ was controlled based on pCO₂ sensor measurements.

### Perfusion Culture

Continuous culture systems were used for this study of combined pCO₂ and culture glucose effects as these can be operated without the inherent variability of batch cultures. This approach should allow a steady-state response to be determined. As shown in Figure 2, the pH and osmolality were kept constant at 6.93 ± 0.02 and 311 ± 4 mOsm/kg, respectively (mean of 56 pseudo-steady state data ±SD).

The cell specific feed rate was 164.6 ± 6.6 μL/10⁶ cells day. Using a stirred tank perfusion bioreactor at 10⁷ cells/mL makes these results relevant to high cell density production cultures, where CO₂ accumulation can be a problem. It was not practical to operate multiple perfusion cultures in parallel, so a sequential experimental design was selected. One challenge for this approach was to verify that the results were not significantly influenced by the sequential culture conditions. Therefore, the perfusion culture was returned to a standard 50 mmHg pCO₂ level after exploring the response at 140 and 220 mmHg pCO₂. The CSGUR, CSLPR and CSIgGPR all returned to within 10% of the original values, both for high glucose and glucose-limited conditions. These standard pCO₂ level replicate data are provided in the plots of data below and confirm the results.

### Effects of Glucose Concentration

Changing the feed medium from M1 (33 mM glucose) to M2 (6 mM glucose) led to a drop in the residual culture glucose from 14.3 ± 0.8 mM (mean of 28 pseudo-steady

### Table I. Cell specific parameters in CHO cell batch culture at different CO₂ gas inlet concentrations.

<table>
<thead>
<tr>
<th>CO₂ gas inlet concentration</th>
<th>CSIgGPR (pg/cell/day)</th>
<th>CSGUR (nmol/10⁶ cells/h)</th>
<th>CSLPR (nmol/10⁶ cells/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% CO₂</td>
<td>17.7 ± 2.7</td>
<td>196 ± 10</td>
<td>262 ± 58</td>
</tr>
<tr>
<td>20% CO₂</td>
<td>17.8 ± 1.4</td>
<td>208 ± 10</td>
<td>265 ± 54</td>
</tr>
<tr>
<td>40% CO₂</td>
<td>12.8 ± 3.6</td>
<td>66 ± 14</td>
<td>113 ± 24</td>
</tr>
</tbody>
</table>

Cell specific IgG production rate (CSIgGPR), cell specific glucose uptake rate (CSGUR) and cell specific lactate production rate (CSLPR) represent the average ±SEM from the day-to-day specific rates between days 4 and 6 (for CSIgGPR; 2 data) or days 3 and 6 (for CSGUR or CSLPR; three data).

**Figure 2.** Time profiles of CHO cell culture in a perfusion reactor. Osmolality (○) and pH (□) were kept constant, while pCO₂ level (▲) was twice increased from -50 to -140 mmHg and -220 mmHg, and returned back to -50 mmHg. Glucose concentration in the feed medium changed from 33 to 6 mM at day 49. Cell specific medium feed rate (○) was kept constant. The top set of arrows represent the pseudo-steady state of each condition.

**Figure 3.** Time profiles of glucose (○), lactate (■) and ammonia (○) concentrations in a perfusion culture. Glucose concentration in the feed medium (line) changed from 33 to 6 mM at day 49. The top set of arrows represent the pseudo-steady state of each condition.
states ±SD) to <0.1 mM (Fig. 3). Hence, the first period is referred to as the ‘high glucose condition’, and the second period as the ‘glucose-limited condition’. The CSGUR under glucose-limited conditions (41.5 ± 1.8 nmol/10^6 cells h) was reduced to one-third of that under high glucose conditions (129 ± 8 nmol/10^6 cells h, Fig. 4), regardless of the pCO₂ level. Also, the lactate concentration fell from 29.4 ± 1.6 mM to between 5 and 11 mM (Fig. 3). The CSAPR was doubled under glucose-limited conditions (from 8.9 ± 0.9 to 17.2 ± 1.4 nmol/10^6 cells h, Fig. 4). Under the glucose-limited condition and up to ~140 mmHg pCO₂, even though the CSGUR was greatly reduced, the growth rate was increased (Fig. 5). This included at ~50 mmHg pCO₂ where an average 26% higher growth rate (P < 0.05) was obtained under the glucose-limited condition. The calculated death rates (k₅) were independent of the glucose concentration (Fig. 5). For glucose-limited relative to the high glucose conditions (Fig. 6), at ~50 mmHg pCO₂ the CSIgGPR was lowered slightly (8%, P < 0.05), not significantly at ~140 mmHg pCO₂ and 15% (P < 0.01) at ~220 mmHg pCO₂. Overall, in all replicate experiments, four out of six steady state data had significantly higher

Figure 4. (a) Time profiles of cell specific parameters in a perfusion culture. Changes of cell specific glucose uptake rate (CSGUR, ◦), lactate production rate (CSLPR, □) and ammonia production rate (CSAPR, ○). The top set of arrows represent pseudo-steady state of each condition. (b) Effects of pCO₂ on cell specific parameters under high glucose (14.3 ± 0.8 mM, closed symbols) and glucose-limited (<0.1 mM, open symbols) conditions. CSGUR ( ◦), CSLPR ( □) and the yield of lactate produced vs. glucose uptake ( ◇). The plot represents the mean of six data from each pseudo-steady state and the bar represents the SEM.

Figure 5. (a) Time profiles of calculated cell growth rate ( ◦) and cell viability ( □) in a perfusion culture. The top set of arrows represent the pseudo-steady state of each condition. (b) Effects of pCO₂ on calculated cell growth ( ◦) and death ( □) rates under high glucose (14.3 ± 0.8 mM, closed symbols) and glucose-limited (<0.1 mM, open symbols) conditions. The plot represents the mean of six data from each pseudo-steady state and the bar represents the SEM.
Effects of $pCO_2$

Both the CSGUR and CSAPR were not significantly affected by $pCO_2$, whereas the CSLPR increased as $pCO_2$ increased (Fig. 4b). The yield of lactate on glucose increased from 1.1 to 1.7 as the $pCO_2$ increased from $\sim$50 to $\sim$220 mmHg ($P < 0.01$) under the glucose-limited condition, whereas there was little increase ($P < 0.05$) under the high glucose condition.

Under the high glucose condition, the specific growth rate declined with increasing $pCO_2$ (Fig. 5b; from 0.021 at $\sim$50 mmHg $pCO_2$ to 0.009 h$^{-1}$ at $\sim$220 mmHg $pCO_2$). Under the glucose-limited condition, a higher growth rate was obtained at lower $pCO_2$ levels, (interestingly, 70% higher at $\sim$140 mmHg $pCO_2$), but it dropped drastically under the $\sim$220 mmHg condition to 0.008 h$^{-1}$, approximately the level of the high glucose condition. Although the $k_d$ was not affected by $pCO_2$, the viability dropped as $pCO_2$ increased (Fig. 5a), and this was attributed to the decreasing ratio of $\mu$ to $k_d$ with increasing $pCO_2$ (Fig. 5b). There was a 14% decrease of the CSIgGPR under the glucose-limited condition between $\sim$50 and $\sim$220 mmHg ($P < 0.01$), whereas the CSIgGPR dependence on $pCO_2$ was less clear at high glucose (Fig. 6). The non-monotonic response to $pCO_2$ is similar to that reported by deZengotita et al. (2002). Overall, any decreasing trend in CSIgGPR, as a function of $pCO_2$, was small.

There have been a number of reports about $pCO_2$ effects on cell cultures (Schmelzer and Miller, 2002), but the effects are often cell type or product dependent; furthermore, few reports have given explanations for the observed effects. More recently, deZengotita et al. (2002) reported that the intracellular pH ($pHi$) in hybridoma cells was lower by 0.1–0.2 pH units at higher (140–250 mmHg) $pCO_2$. The

![Figure 6. Effect of $pCO_2$ on cell specific IgG production rate under high glucose (14.3 ± 0.8 mM, ○) and glucose-limited (<0.1 mM, □) conditions. The plot represents the mean of six data from each pseudo-steady state and the bar represents the SEM.](image_url)
CSGUR of their hybridoma cells decreased significantly with increasing pCO₂ (from 600 nmol/10⁶ cells h at 40 mmHg to 200 nmol/10⁶ cells h at 250 mmHg with a similar drop in CSLPR), when glucose was not limited. They suggested that glycolysis could be inhibited at higher pCO₂ because low pHᵢ inhibits phosphofructokinase, (a rate-limiting enzyme in glycolysis, Belt et al., 1979; Fidelman et al., 1982) and also because pHᵢ can affect the localization of hexokinase (Miccoli et al., 1996). Our CHO results were not entirely consistent with these observations, since there was no dependence of CSGUR on pCO₂ (Fig. 4b). Perhaps this inconsistency between these results was due to the different cell types employed in each study. In the deZengotita (2002) report, the pHᵢ change appeared not to be the only reason for the effects of increased pCO₂, since glycolysis continued to decrease as pCO₂ increased, even though there was no further decrease in pHᵢ.

Effects on Amino Acid Metabolism

To further evaluate cell physiology changes, cell specific amino acid consumption or production rates were measured and calculated (Table II). The results under the high glucose conditions were similar to the data from Ozturk and Palsson (1991a). Glutamine was the most consumed amino acid, but there was no clear dependency of the cell specific consumption rate on either the glucose concentration or the pCO₂. The alanine production was low or negligible under the glucose-limited conditions. On the other hand, alanine was produced under the high glucose conditions, and its rate of production decreased as the pCO₂ was increased. These changes, combined with the CSAPR changes, indicate different metabolism of glutamate under the two conditions.

Under high glucose conditions, after glutamine is converted to glutamate, it was mostly transaminated with pyruvate to produce alanine and α-ketoglutarate. Under glucose-limited conditions, the glutamate appears to have been mostly deaminated to produce α-ketoglutarate and ammonia, consistent with the decreased alanine production rate (Table II) and the increased CSAPR (Fig. 4a) under the glucose-limited condition. Theoretically, more ATP is available via deamination of glutamate followed by the complete oxidation of α-ketoglutarate (27 mol of ATP generated/mol of glutamate consumed) than via transamination (9 mol of ATP) (Altamirano et al., 2001a). This may help explain the higher growth rates at lower pCO₂ under glucose-limited conditions (Fig. 5). However, when pCO₂ was increased to ~220 mmHg with glucose limitation, the flux from pyruvate to lactate increased (Fig. 4b), apparently decreasing the flux from pyruvate to the TCA cycle. This may contribute to the dramatic decrease of the growth rate at high pCO₂ (Fig. 5). Under these conditions, it may be that pyruvate decarboxylase activity, which catalyzes the conversion of pyruvate to acetyl-CoA, was reduced by the high concentration of CO₂, since it is also a product of the reaction.

Effects on IgG Glycosylation

The molecular characterization of post-translational modifications is an important aspect of the development of a monoclonal antibody for therapeutic use (Müthing et al., 2003). Figure 7 shows an example of the sugar mapping profile of the IgG produced. IgG has a biantennary complex type oligosaccharide on each heavy chain, and each branch of the oligosaccharide can be galactosylated. Therefore, the released oligosaccharides were separated into four peaks based on their galactose content and positions. The sugar mapping profile was significantly affected by the glucose concentrations (Fig. 8). The relative G(0) peak area was higher under the high glucose conditions compared to the glucose-limited conditions (P < 0.01). Meanwhile the relative G(1)-1, G(1)-2 or G(2) peak areas under the high glucose conditions were lower than under the glucose-limited conditions (P < 0.01 for G(1)-1 and G(1)-2, P < 0.05 for G(2)). On the other hand, the sugar mapping profiles in Figure 8 did not reveal a consistent dependence on the pCO₂.

Figure 7. Example of the sugar mapping profile of the produced IgG. Peaks were assigned as; G(0): agalacto biantennary complex type oligosaccharide, G(1)-1: monogalacto on the α₁,6 branch, biantennary complex type oligosaccharide, G(1)-2: monogalacto on the α₁,3 branch, biantennary complex type oligosaccharide, G(2): digalacto biantennary complex type oligosaccharide. The y-axis represents the output from the fluorescence detector.
The galactosylation pattern of monoclonal antibody produced by hybridoma cells was reported to be altered by the culture pH (Müthing et al., 2003), and by oxygen levels (Kunkel et al., 1998). On the other hand, Moran et al. (2000) reported that the distribution of glycoforms of the IgG1 produced by recombinant murine myeloma cells was not affected by the varying process control conditions within certain ranges of temperature and oxygen concentration. Cruz et al. (2000) concluded that metabolic shifts do not influence the glycosylation patterns of a recombinant protein expressed in BHK cells, although they found a slight increase in terminal galactosylation with the decrease in glucose concentration. Our results supported that there can be minor changes in galactosylation resulting from glucose limitation. Limited glucose might have led to an increase in the proportion of UDP-galactose to UDP-glucose in the UDP-hexose pool, which can lead to slightly higher galactosylation in NS0 cells (Hills et al., 2001).

**Conclusion**

The combined effects of culture glucose concentration and pCO$_2$ were investigated. A clear metabolic shift was observed when CHO cells were transferred from high glucose to glucose-limited conditions. An interesting interaction between these variables was that the growth rate was 1.7-fold higher under glucose-limited conditions at a pCO$_2$ level of $\sim$140 mmHg, probably because of the change of metabolic balance, including due to changes in the lactate formation and glutamine metabolic pathway. However, when the pCO$_2$ was increased to $\sim$220 mmHg, the ratio of lactate to glucose increased from 1.1 to 1.7 and the growth rate dropped to the level obtained for the high glucose condition. Increased galactosylation was also observed under the glucose-limited conditions. It is clear that both glucose limitation and pCO$_2$ levels need to be carefully considered when optimizing mammalian cell culture processes.

**Figure 8.** Effects of pCO$_2$ on the sugar mapping profile of produced IgG under high glucose (14.3 ± 0.8 mM, •) and glucose-limited (<0.1 mM, ○) conditions.
We acknowledge Dr. R. Spokane of YSI for his technical assistance and for the loan of the CO₂ measurement system and C. Glover (UBC) for assistance with the statistical analysis of the data.

References


