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Effect of hypoosmotic pressure on cell growth and antibody production in recombinant Chinese hamster ovary cell culture

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Abstract

To determine the response of recombinant Chinese hamster ovary (rCHO) cells subjected to hypoosmotic pressure, rCHO cells (CS13*–1.0) producing a chimeric antibody were cultivated in the hypoosmolar medium resulting from NaCl subtraction. At hypoosmotic pressure, CS13*–1.0 cells displayed decreased specific growth rate (μ) and increased specific antibody productivity (q_{Ab}). When the medium osmolality was decreased from 300 mOsm kg^{-1} (physiological osmolality) to 150 mOsm kg^{-1}, μ was decreased by 68% and q_{Ab} was increased by 128%. To understand the mechanism of enhanced q_{Ab} resulting from hypoosmotic pressure, cellular responses of cells in the exponential phase of growth were observed at the transcription level. Total cytoplasmic RNA content per cell at 150 mOsm kg^{-1} was increased by 140%, compared with that at 300 mOsm kg^{-1}. On a per μg RNA basis, immunoglobulin (Ig) mRNA levels at 150 mOsm kg^{-1} were comparable to those at 300 mOsm kg^{-1}, indicating that hypoosmotic pressure did not lead to the preferential transcription of Ig mRNAs. Taken together, the data obtained here suggest that the increase in total RNA pool is primarily responsible for the enhanced q_{Ab} of CS13*–1.0 cells subjected to hypoosmotic pressure.

Introduction

Osmolality is one of the most important physical properties of culture media. Most cell culture media are designed to have an osmolality of 280–320 mOsm kg^{-1}, which is known to be quite acceptable for most cells (Freshney, 1994). As the osmolality goes far from this standard range, hybridomas, transfectomas, and recombinant Chinese hamster ovary (rCHO) cells showed some obvious changes in cell size, nutrient transport, cellular synthesis, specific growth rate (μ) and specific antibody productivity (q_{Ab}). Among these changes, the most important changes for cell culturist are probably those in μ and q_{Ab}.

When subjected to hyperosmotic pressure, hybridomas (Lee and Park, 1995; Oh et al., 1993, 1995; Ozurk and Palsson, 1991; Øyaas et al., 1994a, b; Reddy and Miller, 1994), transfectomas (Lee and Lee, 2000), and rCHO cells (Kim et al., 2000; Ryu et al., 2000) display increased q_{Ab} and decreased μ. Hence, the enhanced q_{Ab} during batch culture does not result in a substantial increase in the final antibody concentration due to the depressed cell growth at higher osmolality (Lee and Park, 1995; Oh et al., 1993, 1995; Ozurk and Palsson, 1991; Øyaas et al., 1994a, b; Reddy and Miller, 1994). Because of the potential of commercial strategies based on hyperosmotic pressure induced by addition of cheap salts to media, physiological responses of hybridomas, transfectoma, and rCHO cells to hyperosmotic pressure have been extensively studied. As a result, various strategies to overcome depressed cell growth at elevated osmolality were developed. They are the adaptation of cells to hyperosmolality (Lin et al., 1999; Oh et al., 1993), the use of osmoprotective compound like glycine betaine (Øyaas et al., 1994a, b; Ryu et al., 2000) and the immobilized cell culture where cell growth and antibody production stages are separated (Park and Lee, 1995).

Physiological responses of hybridomas to hypoosmotic pressure have been studied (Ryu and Lee, 1997). Furthermore, the hypoosmotic pressure has been successfully applied to fed-batch culture of hybridoma cells for improvement of culture longevity. The use of hypoosmolar medium as an initial medium in fed-batch culture improved culture longevity of S3H5/γ2bA2 hybridoma, resulting in a substantial increase in a final antibody titer (Ryu and Lee, 1999).

While much work has been performed on the response of rCHO cells to hyperosmotic pressure (Chen et al., 1998; Kim et al., 2000; Ryu et al., 2000; Takagi et al., 2000), there are, to date, few reported studies on the response of rCHO cells to hypoosmotic pressure. Considering the importance of rCHO cells as a popular mammalian host for the commercial production of therapeutically important proteins (Hayter et al., 1991; Kaufman et al., 1983; Wurm et al., 1996), physiological responses of rCHO cells to hypoosmotic pressure need to be studied. In this study, we investigated the responses of rCHO cells producing chimeric antibody (CS13*–1.0) to hypoosmotic pressure in regard to μ and q_{Ab}. Furthermore, to understand the effect of hypoosmotic pressure on q_{Ab} of rCHO cells at the basic cellular levels, possible changes in Ig mRNA levels caused by hypoosmotic pressure were examined.

Materials and methods

Cell line, medium, and culture maintenance

The rCHO cells (CS13*–1.0) expressing a chimeric antibody directed against hepatitis B virus (HBV) were used in this study, and their establishment was described previously (Kim et al., 1998). Briefly, they were established by transfection of the light chain (LC) and heavy chain (HC) expression plasmids into dihydrofolate reductase (DHFR)-deficient CHO cells (DG44) and subsequent dhfr/methotrexate (MTX)-mediated gene amplification. The rCHO cells (CS13*–1.0) were selected at 1.0 μM MTX.

The medium for culture maintenance was α-minimal essential medium (α-MEM, Gibco Laboratories, Grand Island, NY) supplemented with 10% dialyzed fetal bovine serum (dFBS, Gibco), and 1.0 μM of MTX. The cells were maintained as monolayer cultures in 75 cm^2 T-flasks (Bellco Glass, Vineland, NJ) in a humidified 5% CO_2 incubator at 37 °C. The cells were passed every 3 to 4 days upon reaching confluency.

Hypoosmolar culture media with various osmolalities were prepared by reducing NaCl concentration in the standard medium, while the concentrations of all other medium components were kept constant. The standard medium with physiological osmolality was α-MEM supplemented with 10% dialyzed fetal bovine serum (dFBS, Gibco), and 1.0 μM of MTX. The osmolalities of standard and hypoosmolar media were 300, 250, 200, and 150 mOsm kg^{-1}.

Batch culture

Exponentially growing cells in a standard α-MEM/10% dFBS were inoculated into 6 well plates containing 5 ml of media with various osmolalities. The initial viable cell concentration was approximately 1×10^5 cells ml^{-1}. One well was sacrificed every 24 hr for determination of cell concentration. The supernatant was aliquoted and kept frozen at –20 °C for antibody, glucose and lactate assays. The cultures were performed in a humidified 5% CO_2 incubator at 37 °C. Experiments were repeated three times.

Analytical methods

Cell concentration was estimated using a hemacytometer. Viable cells were distinguished from dead cells by the trypan blue dye-exclusion method. The supernatant was aliquoted and kept frozen at –20 °C for later analyses. Osmolality was measured using an osmometer (Automatic semi-micro osmometer, model A0300, Knauer, Berlin). Glucose and lactate concentrations were measured using a glucose/lactate analyzer (Model 2300 STAT, Yellow Springs Instruments, Yellow Springs, OH). The secreted chimeric antibody demonstrating anti-S specificity was measured by enzyme-linked immunosorbant assay (ELISA) as described previously (Hong et al., 1992; Kim et al., 1996).

Evaluation of specific growth, consumption, and production rates

The specific growth rate (μ), specific glucose consumption rate (q_{glc}), specific lactate production rate (q_{lac}) and specific antibody productivity (q_{Ab}) were based on data collected during the exponential growth phase and were evaluated as described earlier (Lee et al., 1991).

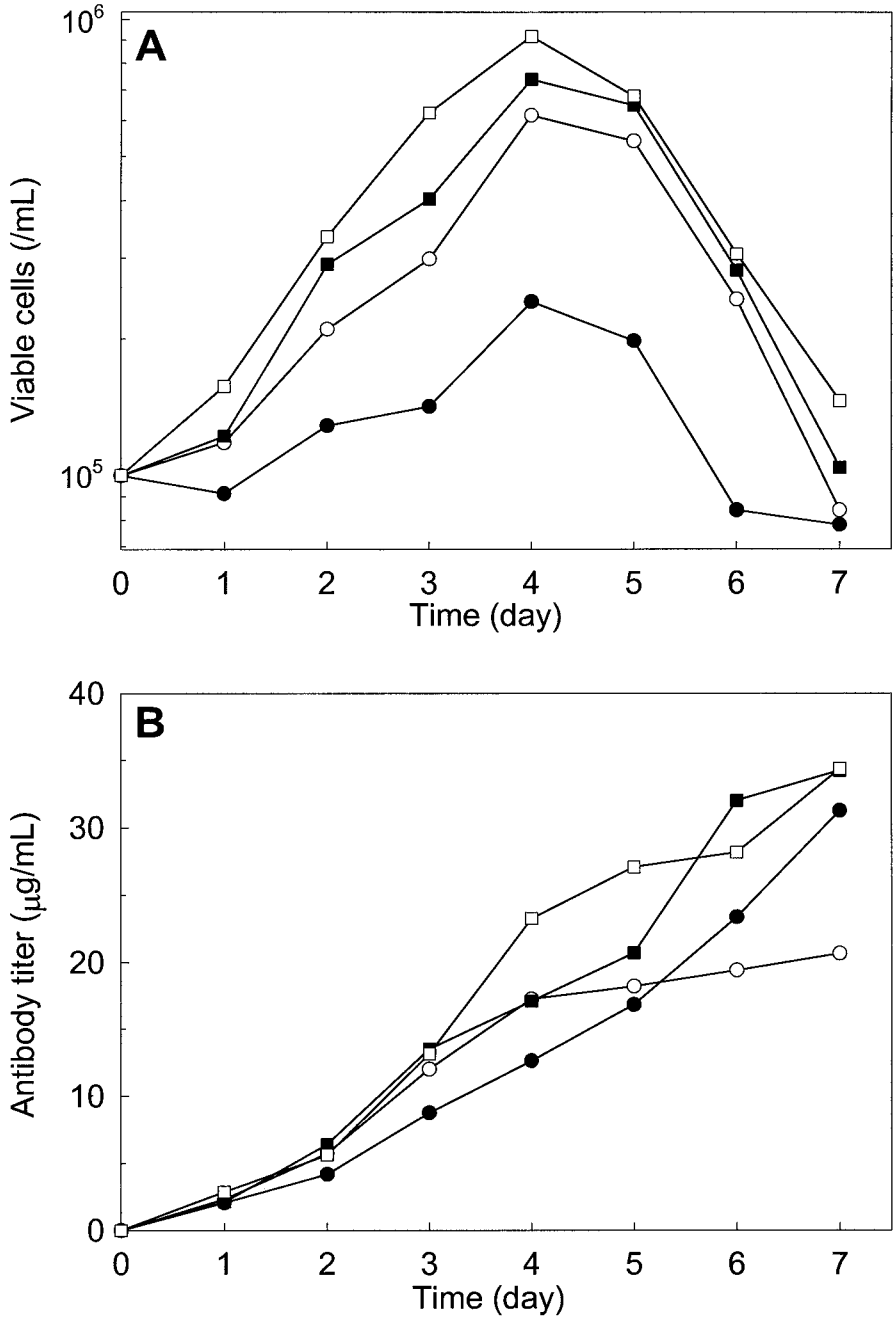

Figure 1. Batch cultures of CS13*–1.0 rCHO cells at various osmolalities. Viable cell concentration (A) and antibody concentration (B). (●) 150 mOsm kg^{-1}; (○) 200 mOsm kg^{-1}; (■) 250 mOsm kg^{-1}; (□) 300 mOsm kg^{-1}.

Table 1. Effect of hypoosmotic pressure on μ, q_{Ab}, maximum viable cell concentration, maximum antibody concentration, q_{glc}, q_{lac}, and $Y_{lac/glc}$ in batch cultures[a]

Medium osmolality (mOsm kg^{-1})	μ (day^{-1})	q_{Ab} ($\mu g\ 10^{-6}$ cells day^{-1})	Max viable cell concentration (10^6 cells ml^{-1})	Max antibody concentration ($\mu g\ ml^{-1}$)	q_{glc} ($\mu mol\ 10^{-6}$ cells day^{-1})	q_{lac} ($\mu mol\ 10^{6}$ cells day^{-1})	$Y_{lac/glc}$ (mol mol^{-1})
150	0.19±0.11	31.93±3.15	0.24±0.07	31.27±0.35	5.26±0.37	9.01±0.63	1.87±0.07
200	0.46±0.02	23.77±2.10	0.62±0.02	20.66±0.16	5.24±0.37	8.17±1.80	1.56±0.26
250	0.58±0.06	18.75±0.14	0.74±0.07	34.24±1.29	4.42±0.26	7.43±0.83	1.68±0.11
300	0.60±0.03	14.02±0.42	0.92±0.04	34.68±0.41	2.46±0.02	4.10±0.32	1.67±0.09

[a] Mean ± SD. This experiment was performed three separate times.

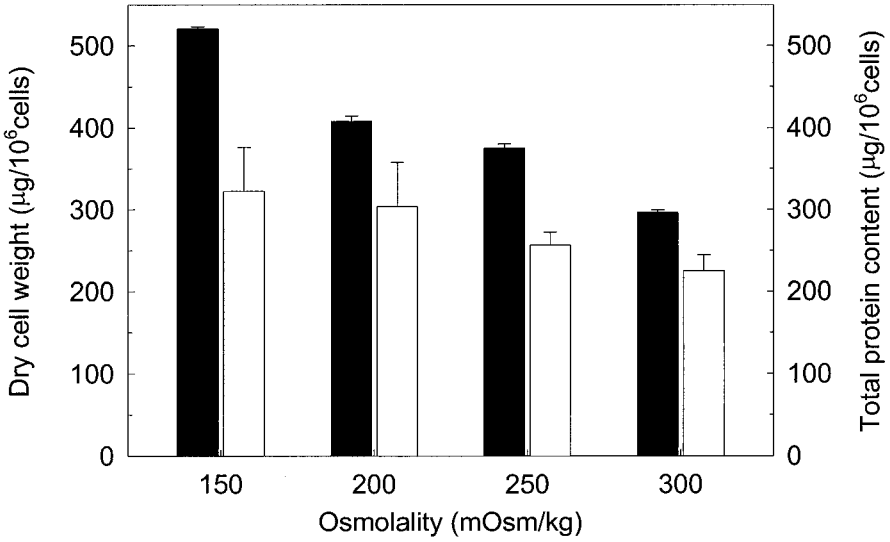

Figure 2. The effect of hypoosmotic pressure on DCW (■) and total intracellular protein content (□).

Table 2. Effect of hypoosmotic stress on total cytoplasmic RNA content and relative transcription levels of HC and LC mRNAs of exponentially growing rCHO cells[a]

Medium osmolality (mOsm kg^{-1})	Total cytoplasmic RNA content ($\mu g\ 10^{-6}$ cells)	Relative total RNA content[b]	Relative transcription level[b, c]	
			HC mRNA	LC mRNA
150	23.40±0.90	2.40±0.09	2.62±0.27	2.83±0.08
200	21.91±3.03	2.25±0.31	2.23±0.04	2.57±0.07
250	17.61±2.38	1.81±0.24	1.92±0.42	1.99±0.01
300	9.73±1.08	1	1	1

[a] Mean ± SD. This experiment was performed three separate times.
[b] The values at various osmolalities were normalized by those at standard medium (300 mOsm kg^{-1}).
[c] The relative mRNA levels were quantified on the basis of cells.

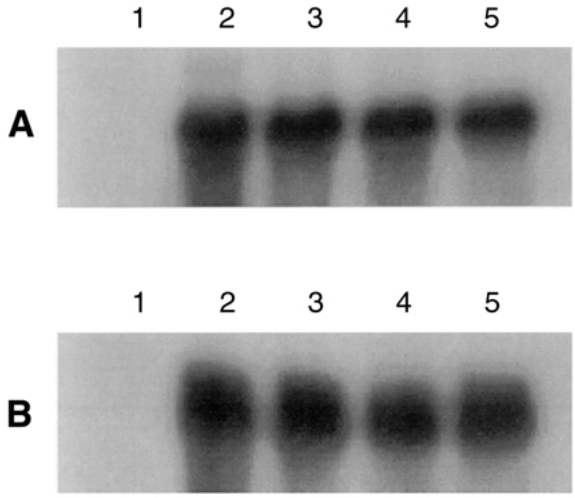

Figure 3. Northern blot analysis of CS13*–1.0 rCHO cells at various osmolalities. Heavy chain (A) and light chain (B). Lane 1, DG44 as a negative control; lane 2–5, CS13*–1.0 cells (150, 200, 250, 300 mOsm kg^{-1}, respectively).

Dry cell weight and total intracellular protein content

For the determination of dry cell weight (DCW) and intracellular protein content, the cells in the mid-exponential phase of growth were harvested by centrifugation and were washed twice with cold phosphate buffered saline (PBS). Analyses of DCW and intracellular protein content were performed as described previously (Lee and Lee, 2000).

Total cytoplasmic RNA content and Northern blot hybridization

Total cytoplasmic RNA was isolated from cells in the exponential growth phase using the NP-40 method described by Sambrook et al. (1989). The concentration of isolated RNA was determined by measuring the OD_{260} of the sample, assuming that an OD of 1.0 corresponds to 40 μg of single-stranded RNA. Isolated RNA was of high purity, with OD_{260}/OD_{280} ratios over 1.7. After electrophoresis of equal amounts of total cytoplasmic RNA on a 0.8% agarose formaldehyde gel, Northern blot analyses of LC and HC mRNAs were performed as described previously (Ryu et al., 2000).

Results

Cell growth, antibody production, glucose consumption, and lactate production

To determine the effect of hypoosmotic pressure on rCHO cells (CS13*–1.0) in regard to growth, antibody production, glucose consumption, and lactate production, batch cultures with various osmolalities in the range of 150–300 mOsm kg^{-1} were performed three separate times.

Figure 1 shows typical profiles of cell growth and antibody production during batch culture. The osmolality did not change significantly during the culture (data not shown). When subjected to hypoosmotic pressure, cell growth was depressed. The cells could still grow at 200 mOsm kg^{-1}, though their growth and antibody production were decreased. At this osmolality, cell viability remained over 75% during exponential growth phase. When the medium osmolality was further decreased to 150 mOsm kg^{-1}, cell growth was significantly suppressed. The maximum viable cell concentration obtained at 150 mOsm kg^{-1} was only 0.24 $\pm$0.07 (mean $\pm$ standard deviation, n = 3) $\times 10^6$ cells ml^{-1} which is approximately 26% of that at 300 mOsm kg^{-1}. Antibody production was also decreased at 150 mOsm kg^{-1}, but to a lesser extent. The maximum antibody concentration obtained at 150 mOsm kg^{-1} was 31.27 ± 0.35 μg ml^{-1} which is approximately 90% of that at 300 mOsm kg. This result implies that hypoosmotic pressure, like hyperosmotic pressure (Ryu et al., 2000), enhances q_{Ab}. The q_{Ab} at 150 mOsm kg^{-1} was 31.93$\pm$3.15 μg 10^{-6} cells day^{-1}, which is 128% higher than that at 300 mOsm kg^{-1}. Thus, hypoosmotic pressure induced by NaCl subtraction suppresses the growth of CS13*–1.0 cells while it increases their q_{Ab}. The μ, maximum viable cell concentration, q_{Ab}, and maximum antibody concentration at various osmolalities are summarized in Table 1.

Glucose and lactate concentrations were measured during the culture. Glucose utilization was accompanied by a corresponding accumulation of lactate (data not shown). Like q_{Ab}, both q_{glc} and q_{lac} was also increased at hypoosmolalities. Because these metabolic rates increased to a similar extent, the ratio of lactate produced to glucose consumed, $Y_{lac/glc}$, did not change significantly regardless of osmolalities. The q_{glc}, q_{lac}, and $Y_{lac/glc}$ were also summarized in Table 1.

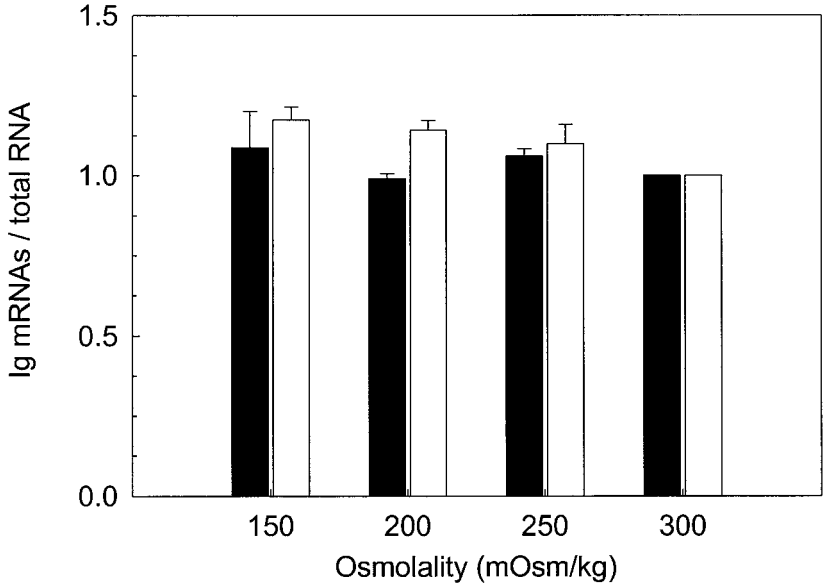

Figure 4. The ratio of Ig mRNAs to total cytoplasmic RNA. (■) heavy chain; (□) light chain. To get Ig mRNA/total RNA, the relative Ig mRNA level (on a per-cell basis) was divided by the relative total RNA (on a per-cell basis) at corresponding osmolalities.

Dry cell weight (DCW) and total intracellular protein content

To determine DCW and total intracellular protein content, cells in the mid-exponential phase of growth were sampled from batch cultures with various osmolalities.

Figure 2 shows DCW and intracellular protein content at various osmolalities. When subjected to hypoosmotic pressure, DCW was increased. The DCW at 150 mOsm kg^{-1} was 520.53±2.32 μg 10^{-6} cells which is 76% higher than that at 300 mOsm kg^{-1}. The changes in intracellular protein content caused by hypoosmotic pressure were similar to those in DCW. Thus, the ratio of intracellular protein to dry cell weight remained almost constant at 0.70±0.06.

Total cytoplasmic RNA and Ig mRNA levels

To understand the effect of hypoosmotic pressure on q_{Ab} of rCHO cells at the basic cellular levels, possible changes in Ig mRNA levels caused by hypoosmotic pressure were examined. Cells in the mid-exponential phase of growth were sampled from batch cultures with various osmolalities.

Total cytoplasmic RNA content was measured at various osmolalities. No gross degradation of RNA was detected by an electrophoretic method utilizing formaldehyde (data not shown). When subjected to hypoosmotic pressure, total cytoplasmic RNA was increased. At 150 mOsm kg^{-1}, the total cytoplasmic RNA content was increased by 140%, compared with that at 300 mOsm kg^{-1}. Total cytoplasmic RNA content at various osmolalities are summarized in Table 2.

To determine the relative levels of HC and LC mRNAs in the total cytoplasmic RNA, Northern blotting analyses were carried out. Equal amounts of total cytoplasmic RNA were electrophoresed. The total cytoplasmic RNA of exponentially growing DG44 cells was used as a negative control.

Figure 3 shows the autoradigrams obtained as a hybridization of HC and LC probes to the total cytoplasmic RNA. DG44 cells used as a negative control did not show any detectable hybridization signal. On a per-total cytoplasmic RNA basis, the HC mRNA level was kept almost constant, and the LC mRNA level was slightly increased at hypoosmolalities. Thus, hypoosmotic pressure did not induce preferential transcription of Ig mRNAs. When the relative mRNA levels were calculated on a per-cell basis, the relative HC and LC mRNAs levels at 150 mOsm kg^{-1} were increased by 162 and 183%, respectively. This result suggests that the enhanced q_{Ab} of CS13*–1.0 cells subjected to hypoosmotic pressure mainly resulted from the increase in total RNA pool. The relative transcription levels of HC and LC of cells in the exponential phase of growth are also summarized in Table 2.

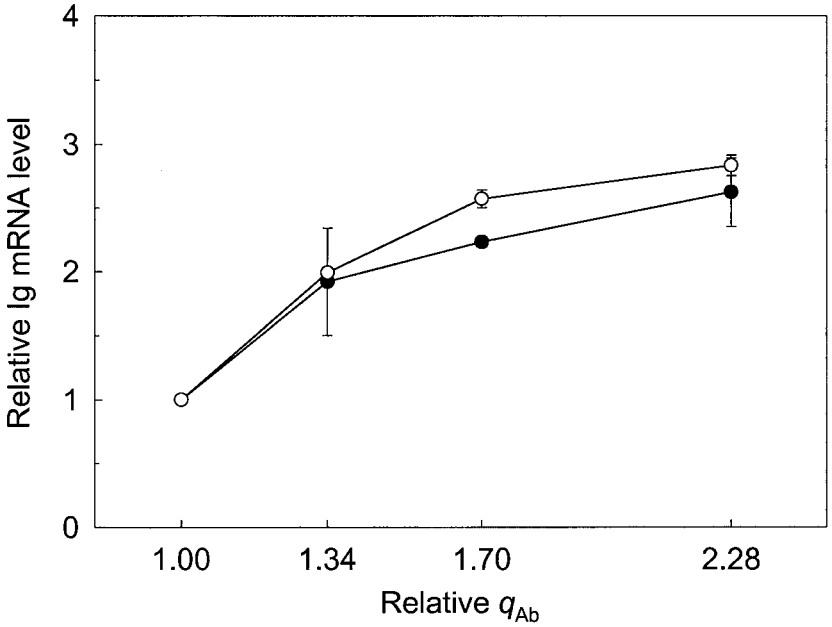

Figure 5. The relationship between relative transcription level of Ig mRNA and relative q_{Ab}. (●) heavy chain; (○) light chain.

Discussion

Hyperosmotic pressure, which can be induced by addition of cheap salts to media, has been suggested as an economical solution to increase antibody production in rCHO cell cultures. When subjected to hyperosmotic pressure, rCHO cells displayed increased q_{Ab} and decreased μ. Furthermore, transcriptional regulation was found to be responsible in part for q_{Ab} enhancement of rCHO subjected to hyperosmotic pressure.

While much work have been performed on the effect of hyperosmotic pressure on rCHO cells, there are, to date, few reported studies on the effect of hypoosmotic pressure on rCHO cells. In an effort to increase antibody production from rCHO cells, the physiological response of rCHO cells (CS13*–1.0) to hypoosmotic pressure resulting from NaCl subtraction from media was investigated.

Like hyperosmotic pressure, hypoosmotic pressure also increased q_{Ab} and decreased μ of rCHO cells. This response of rCHO cells in regard to q_{Ab} is in contrast with that of hybridoma cells. When subjected to hypoosmotic pressure, S3H5/γ2bA2 and DB9G8 hybridomas did not display enhanced q_{Ab} at hypoosmotic pressure (Ryu and Lee, 1997). Thus, the response of mammalian cells to hypoosmotic stress in regard to q_{Ab} appears to vary among cell lines.

Dry cell weight of CS13*–1.0 cells increased at hypoosmotic pressure. This increased cell biomass at hypoosmotic pressure may be due to the enhanced transport of nutrients, particularly amino acids, as at hyperosmotic pressure (Christensen, 1984; Oh et al., 1993, 1995). Thus, feeding of excess nutrients into the cells could lead to an increase in total cytoplasmic RNA, cellular protein, and cell mass. This increased cell mass may influence the way that specific metabolic rates are interpreted, because these rates are calculated based on cell numbers, not based on cell mass. When the medium osmolality was decreased from 300 mOsm kg^{-1} to 150 mOsm kg^{-1}, q_{Ab} of CS13*–1.0 cells was increased by 128%. On the other hand, total cytoplasmic RNA, intracellular protein and DCW was increased by 140, 44, and 76%, respectively. Thus, it is likely that increased total cytoplasmic RNA is primarily responsible for enhanced q_{Ab}.

The detailed mechanism of enhanced q_{Ab} resulting from hypoosmotic pressure has not been investigated at the transcription level. As shown in Figure 4, the hypoosmotic pressure did not substantially change the ratio of Ig mRNAs to total cytoplasmic RNA, suggesting that the hypoosmotic pressure did not induce preferential transcription of Ig mRNAs. Thus, the enhanced q_{Ab} resulting from hypoosmotic pressure could be achieved primarily because the increase in total RNA pool lead to the increased transcription of Ig

mRNA. This relationship between transcription levels of Ig mRNAs and q_{Ab} is shown in Figure 5.

The rCHO cells could grow at 200 mOsm kg^{-1} and displayed enhanced q_{Ab}. This result suggests that the potential use of hypoosmolar medium in a fed-batch culture in order to maximize cell culture longevity. The culture longevity in a fed-batch culture is often limited by the elevated medium osmolality caused by repeated nutrient feeding (Bibila and Robinson, 1995; Bibila et al., 1994). Although S3H5/γ2bA2 hybridoma cells did not show enhanced q_{Ab} at hypoosmotic pressure (Ryu and Lee, 1997), the use of hypoosmolar medium as an initial medium in fed-batch culture improved culture longevity of S3H5/γ2bA2 hybridoma cells, resulting in a substantial increase in the final antibody concentration. Accordingly, it is likely that the use of hypoosmolar medium improves the culture longevity in a fed-batch culture of rCHO cells and thereby, increases the antibody production.

In conclusion, when subjected to hypoosmotic pressure, rCHO cells (CS13*–1.0) displayed decreased μ and increased q_{Ab}. At hypoosmotic pressure, the extent of increase in total cytoplasmic RNA content was similar to that in q_{Ab}. In addition, the hypoosmotic pressure did not induce preferential transcription of IgG mRNAs. Thus, the enhanced q_{Ab} subjected to hypoosmotic pressure is primarily due to the increased total cytoplasmic RNA pool.

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References

Bibila TA, Ranucci CS, Glazomitsky K, Buckland BC & Aunins JG (1994) Monoclonal antibody process development using medium concentrates. Biotechnol Prog 10: 87–96.

Bibila TA & Robinson DK (1995) In pursuit of the optimal fed-batch process for monoclonal antibody production. Biotechnol Prog 11: 1–13.

Chen Z, Liu H & Wu B (1998) Hyperosmolality leads to an increase in tissue-type plasminogen activator production by a Chinese hamster ovary cell line. Biotechnol Tech 12: 207–209.

Christensen HN (1984) Organic ion transport during seven decades: the amino acids. Biochem Biophys Acta 779: 225–269.

Freshney RI (1994) Culture of Animal Cells, 3rd edn., Wiley-Liss, Inc., New York.

Hayter PM, Curling EM, Baines AJ, Jenkins N, Salmon I, Strange PG & Bull AT (1991) Chinese hamster ovary cell growth and interferon production kinetics in stirred batch culture. Appl Microbiol Biotechnol 34: 559–564.

Hong HJ, Kim AK, Ryu CJ, Park SS, Chung HK, Kwon KS, Kim KL, Kim J & Han MH (1992) Cloning and characterization of cDNAs coding for heavy and light chains of a monoclonal antibody specific for pre-S2 antigen of hepatitis B virus. Gene 121: 331–335.

Kaufman RJ, Sharp PA & Latt SA (1983) Evolution of chromosomal regions containing transfected and amplified dihydrofolate reductase sequences. Mol Cell Biol 3: 699–711.

Kim JH, Bae SW, Hong HJ & Lee GM (1996) Decreased chimeric antibody productivity of KR12H-1 transfectoma during long-term culture results from decreased antibody gene copy number. Biotechnol Bioeng 51: 479–487.

Kim SJ, Kim NS, Ryu CJ, Hong HJ & Lee GM (1998) Characterization of chimeric antibody producing CHO cells in the course of dihydrofolate reductase-mediated gene amplification and their stability in the absence of selective pressure. Biotechnol Bioeng 58: 73–84.

Kim TK, Ryu JS, Chung JY, Kim MS & Lee GM (2000) Osmoprotective effect of glycine betaine on thrombopoietin production in hyperosmotic Chinese hamster ovary cell culture: clonal variations. Biotechnol Prog 16: 775–781.

Lee MS & Lee GM (2000) Hyperosmotic pressure enhances immunoglobulin transcription rates and secretion rates of KR12H-2 transfectoma. Biotechnol Bioeng 68: 260–268.

Lee GM & Park SY (1995) Enhanced specific antibody productivity of hybridomas resulting from hyperosmotic stress is cell line-specific. Biotechnol Lett 17: 145–150.

Lee GM, Varma A & Palsson BO (1991) Production of monoclonal antibody using free-suspended and immobilized hybridoma cells: Effect of serum. Biotechnol Bioeng 38: 821–830.

Lin J, Takagi M, Qu Y, Gao P & Yoshida T (1999) Enhanced monoclonal antibody production by gradual increase of osmotic pressure. Cytotechnol 29: 27–33.

Oh SKW, Vig P, Chua F, Teo WK & Yap MGS (1993) Substantial overproduction of antibodies by applying osmotic pressure and sodium butyrate. Biotechnol Bioeng 42: 601–610.

Oh SKW, Chua FKF & Choo ABH (1995) Intracellular responses of productive hybridoma subject to high osmotic pressure. Biotechnol Bioeng 46: 525–535.

Ozturk SS & Palsson B (1991) Effect of medium osmolality on hybridoma growth, metabolism, and antibody production. Biotechnol Bioeng 37: 989–993.

Øyaas K, Ellingsen TE, Dyrset N & Levine DW (1994a) Utilization of osmoprotective compounds by hybridoma cells exposed to hyperosmotic stress. Biotechnol Bioeng 43: 77–89.

Øyaas K, Ellingsen TE, Dyrset N & Levine DW (1994b) Hyperosmotic hybridoma cell cultures: Increased monoclonal antibody production with addition of glycine betaine. Biotechnol Bioeng 44: 991–998.

Park SY & Lee GM (1995) Enhancement of monoclonal antibody production by immobilized hybridoma cell culture with hyperosmolar medium. Biotechnol Bioeng 48: 699–705.

Reddy S & Miller W (1994) Effects of abrupt and gradual osmotic stress on antibody production and content of in hybridoma cells that differ in production kinetics. Biotechnol Prog 10: 165–173.

Ryu JS & Lee GM (1997) Effect of hypoosmotic stress on hybridoma cell growth and antibody production. Biotechnol Bioeng 55: 565–570.

Ryu JS & Lee GM (1999) Application of hypoosmolar medium to fed-batch culture of hybridoma cells for improvement of culture

longevity. Biotechnol Bioeng 62: 120–123.
Ryu JS, Kim TK, Chung JY & Lee GM (2000) Osmoprotective effect of glycine betaine on foreign protein in hyperosmotic recombinant Chinese hamster ovary cell cultures differs among cell lines. Biotechnol Bioeng 70: 167–175.
Sambrook J, Fritsch EF & Maniatis T (1989) Molecular Cloning, 2nd edn., Cold Spring Harbor Laboratory, New York.
Takagi M, Hayashi H & Yoshida T (2000) The effect of osmolarity on metabolism and morphology in adhesion and suspension Chinese hamster ovary cells producing tissue plasminogen activator. Cytotechnol 32: 171–179.
Wurm FM, Petropoulos CJ & O'Connor JV (1996) Manufacture of proteins based on recombinant Chinese hamster ovary cells: Assessment of genetic issues and assurance of consistency and quality, In: ER Schmidt and T Hankeln (eds.), Transgenic Organisms and Biosafety (pp. 283–301), Springer-Verlag, Berlin.