Osmoprotective Effect of Glycine Betaine on Foreign Protein Production in Hyperosmotic Recombinant Chinese Hamster Ovary Cell Cultures Differs among Cell Lines

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Abstract

When 3 recombinant Chinese hamster ovary (rCHO) cell lines, CHO/dhfr-B-22-4, CS13-1.00* and CS13-0.02*, were cultivated in hyperosmolar media resulting from NaCl addition, their specific foreign protein productivity increased with medium osmolality. Glycine betaine was found to have a strong osmoprotective effect on all 3 rCHO cell lines. Inclusion of 15 mM glycine betaine in hyperosmolar medim enabled rCHO cell lines to grow at 557-573 mOsm/kg where they could not grow in the absence of glycine betaine. However, effect of glycine betaine inclusion in hyperomolar medium on foreign protein production differed among rCHO cell lines. CHO/dhfr-B22-4 cells retained enhanced specific human thrombopoietin (hTPO) productivity in the presence of glycine betaine, and thereby, the maximum hTPO titer obtained at 573 mOsm/kg was increased by 72% over that obtained in the control culture with physiological osmolality (292 mOsm/kg). On the other hand, enhanced specific antibody productivity of CS13-1.00* and CS13-0.02* at elevated osmolality decreased significantly in the presence of glycine betaine at a cost of the recovery of cell growth. As a result, the maximum antibody titer at 557 mOsm/kg was similar to that obtained in the control culture with physiological osmolality. Taken together, efficacy of the simultanous use of hyperosmotic pressure and glycine betaine as a means to improve foreign protein production was variable among different rCHO cell lines.

Introduction

Although Chinese hamster ovary (CHO) cells are popular mammalian hosts for the commercial production of therapeutically important proteins[1-3], most of studies on hyperosmotic pressure for improved productivity in mammalian cell culture has been focused on hybridoma cell culture. There are, to date, little studies on the effect of hyperosmotic pressure on rCHO cells.

Here, in order to test the feasibility of using osmoprotective hyperosmolar medium for improved foreign protein production in batch culture of rCHO cells, osmoprotective effects of glycine betaine on cell growth and foreign protein production in hyperosmotic

rCHO cell cultures were investigated.

Materials and Methods

Cell lines. Three different rCHO cell lines used were CHO/dhfr-B22-4, CS13-0.02*, and CS13-1.00*. These cell lines were established by transfection of a foreign gene into dihydrofolate reductase-deficient (dhfr-) CHO cells and subsequent dhfr/methotrexate (MTX)-mediated gene amplification.

Culture maintenance, medium and culture. The medium for culture maintenance of CHO/dhfr-B22-4 was IMDM supplemented with 10% (v/v) dialyzed fetal bovine serum (d-FBS) and 0.08 μM MTX. The media for culture maintenace of CS13-0.02* and CS13-1.00* were α-MEM supplemented with 10% d-FBS and the corresponding levels of MTX. Hyperosmolar culture media with various osmolalities were prepared by addition of NaCl into the standard medium with physiological osmolality. Glycine betaine was added to media with the final concentration of 15mM. Cells exponentially growing in the standard medium were inoculated into 6 well plates. The cultures were performed 3 separate times in a humidified 5% CO_2 incubator at 37℃.

Northern Blot Hybridization. For Northern analysis, total cytoplasmic RNA was isolated from cell lines in mid-exponential phase of growth using the protocol described by Sambrook et al.[4]. Membrane transfer, prehybridization, and hybridization were performed using the protocol as described by Sambrook et al. (1989).

Rusults and Discussion

CHO/dhfr-B22-4

Figure 1 shows cell growth and hTPO production profiles during batch cultures. Cell growth was depressed at higher osmolalities (Fig. 1A). Glycine betaine was found to have osmoprotective effects. The μ and maximum viable cell concentration in hyperosmolar culture with 15mM glycine betaine (494 mOsm/kg) were increased by 46% and 40%, respectively, as compared with the hyperomolar culture without glycine betaine (467 mOsm/kg).

Like most hybridomas, CHO/dhfr-B22-4 cells displayed enhanced q_{hTPO} at elevated osmolalities. However, osmoprotective effect of glycine betaine did not decrease q_{hTPO} significantly at a cost of the recovery of cell growth, which led to the improvement of volumetric productivity of hyperosmotic culture supplemented with glycine betaine.

CS13-1.00*

Figure 2 shows cell growth and antibody production profiles during batch cultures. Cell growth was depressed at elevated osmolality (Fig 2A). Cell growth of CS13-1.00* at

hyperosomolality was improved significantly in the presence of glycine betaine.
Like CHO/dhfr-B22-4, CS13-1.00* cells displayed enhanced q_{Ab} at elevated osmolalities. However, addition of glycine betaine to hyperosmolar culture did not increase the maximum antibody concentration (Fig. 2B). Thus, effect of glycine betaine inclusion in the hyperosmolar medium on improved foreign protein production was found to be cell line-specific.

CS13-0.02*

Different responses of CHO/dhfr-B22-4 and CS13-1.00* to hyperosmotic pressure under osmoprotective conditions may be related to their gene copy numbers. To test this hypothesis, batch cultures of CS13-0.02* with various osmolalities in the range of 305-557 mOsm/kg were carried out.
Similar results to those of CS13-1.00* were obtained regarding cell growth and antibody production in all conditions (data not shown). Glycine betaine exerted osmoprotective effect on cell growth, but did not enhance maximum antibody concentration.

Northern Blot Hybridization

To understand osmoprotective effect of glycine betaine on q of rCHO cells at the transcription level, the relative hTPO mRNA content of CHO/dhfr-B22-4 and Ig mRNA contents of CS13-1.00* and CS13-0.02* were quantified using Northern blot hybridization. On a per cell basis, hTPO mRNA content was decreased slightly by addition of glycine betaine, but was significantly higher than that in a control culture.
Different results were obtained for both CS13-1.00* and CS13-0.02* cells. Ig mRNA content per cell decreased at hyperosmolality in the presence of glycine betaine.
Thus, mRNA contents per cell correlate with q in all three rCHO cell lines, indicating that transcriptional regulation is responsible in part for q at hyperosmolality in the absence as well as the presence of glycine betaine.

References

1. Kaufman, R. J., Sharp, P. A., Latt, S. A. "Evolution of chromosomal regions containing transfected and amplified dihydofolate reductase sequences" (1983), Mol. Cell. Biol., **3**, 699-711
2. Hayter, P. M., Curling, E. M., Baines, A. J., Jenkins, N., Salmon, I., Strange, P. G., Bull, A. T. "Chinese hamster ovary cell growth and interferon production kinetics in stirred batch culture" (1991), Appl. Microbiol. Biotechnol., **34**, 559-564
3. Wurm, F. M., Petropoulos, C. J., O'Connor, J. V. (1996) "Manufacture of proteins based on recombinant Chinese hamster ovary cells: assessment of genetic issues and assurance of consistency and quality, pp. 283-301. In: E. R. Schmidt and T. Hankeln (eds.), Transgenic organisms and biosafety". Springer-Verlag, Berin.

4. Sambrook, J., Fritsch, E. F., Maniatis, T. (1989) "Molecular cloning: a laboratory manual, 2nd edition" Cold Spring Harbor Laboratory Press. Plainview, NY

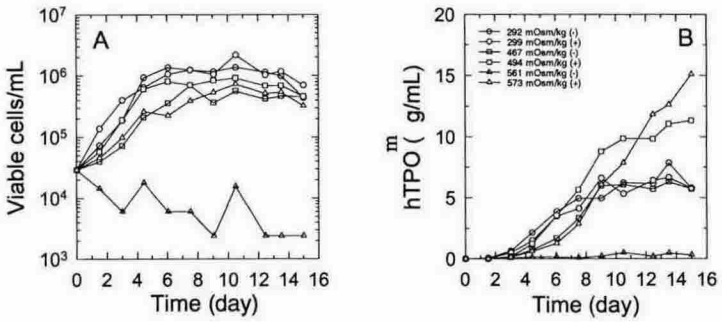

Figure 1. Batch culture of CHO/dhfr-B22-4 cells at various osmolalities in the presence and absence of glycine betaine. Viable cell concentration (A) and hTPO concentration (B). +/- denotes the presence/absence of glycine betaine, respectively.

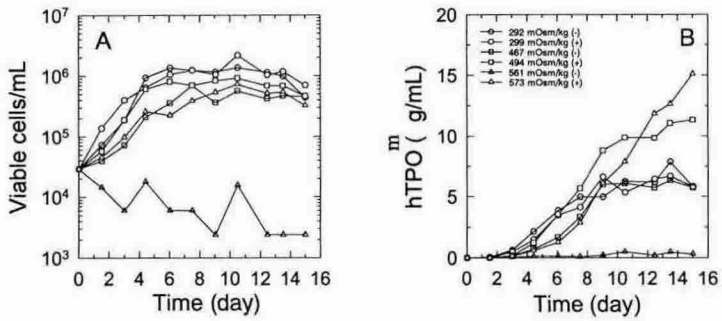

Figure 2. Batch culture of CS13-1.00* cells at various osmolalities in the presence and absence of glycine betaine. Viable cell concentration (A) and antibody concentration (B). +/- denotes the presence/absence of glycine betaine, respectively.