Proteome Analysis of Recombinant CHO Cells Under Hyperosmotic Stress

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ABSTRACT

Under hyperosmotic stress, rCHO cells display decreased specific growth rate (μ) and increased specific antibody productivity (q_{Ab}). The effects of hyperosmotic stress on batch culture cellular dynamics are not well understood. To this end, we conducted a proteome profile of rCHO cells, using 2D-gel, MALDI-TOF-MS and MS/MS. As a result, the proteome profile of rCHO cells could be established using 41 identified proteins. Based on this proteome profile of rCHO cells, we have found at least 8 differently expressed spots at hyperosmotic osmolality (450 mOsm/kg). Among these spots, two metabolic enzymes were found to be up-regulated (pyruvate kinase and GAPDH), while down-regulated protein was identified as tubulin. It shows that hyperosmotic stress can alter metabolic state, by up-regulated activities of two glycolysis enzymes, which could lead to activate the generation of metabolic energy. Tubulin expression was down-regulated, suggesting a reduction of cell division. Finally, the increased conversion energy could leads to improve overall productivity.

INTRODUCTION

Hyperosmotic pressure, which can be induced by addition of cheap salts or sugars to culture media, has been suggested as an economical solution to increase the specific foreign protein productivity q in rCHO cell culture[1) 2)]. However, the enhanced q of rCHO cells in a hyperosmolar batch culture does not result in a substantial increase in the final foreign protein concentration.

To overcome this drawback, extensive efforts have been made regarding cell culture

with limited success. However, studies on intracellular responses of rCHO cells to hyperosmotic pressure have not been fully substantiated as yet, though they could eventually lead to a better insight into possible environmental or genetic manipulation approaches for increasing foreign protein production. 2D-PAGE followed by mass spectrometric analysis is a commonly used method for the identification of proteins in complex mixture. When these technologies are used in concert to the study on cell culture, their impact on foreign protein production can be maximized. To our knowledge, there is, to date, no report on protein pattern of rCHO cells under hyperosmotic pressure. Hence, in this communication, we investigated the protein patterns of rCHO cells expressing a chimeric antibody under hyperosmotic pressure using the proteomic approach.

MATERIALS AND METHODS

Cell line, medium, and culture The rCHO cells (CS13*-1.00) expressing a chimeric antibody directed against hepatitis B virus (HBV) were used in this study. The standard medium was α-MEM supplemented with 10% dFBS, and 1.0μM of MTX. Hyperosmolar culture medium was prepared by adding NaCl stock solution in the standard medium, while the concentrations of all other medium components were kept constant. The osmolalities of standard and hyperosmolar media were 300, 350, 400 and 450 mOsm/kg, respectively.

2-D PAGE Exponentially growing cells were harvested at 72 hrs after seeding and were washed twice with PBS. 2 mg of proteins was loaded onto 17cm ReadyStrip IPG strips. IEF was performed to 50,000 Vhr using PROTEAN IEF System (Bio-Rad). Equilibrated IPG strip was then placed on top of 15% SDS-PAGE gels (16cm*20cm*1.0mm) and then electrophoresed at 45 mA. Silver-stained gel image was analyzed visually and automatically by the PDQuest™ software. For construction of reference gel, abundant spots were excised for further mass spectrometric analysis. After comparing 2D gels in a matchset, protein regulation, caused by hyperosmotic stress, was detected.

MALDI-TOF-MS and MS/MS The resulting tryptic peptides were dissolved in 0.5 % trifluoroacetic acid (TFA) solution, and then desalted using ZipTipC18 (Millipore, Bedford, MA) tip. Peptides were eluted directly onto MALDI target by CHCA matrix solution. All mass spectra were acquired in positive reflection mode of a 4700 Proteomics Analyzer (Applied Biosystems, Framingham, MA). Fragmentation patterns of unidentified protein

spots were analyzed by MS/MS (MALDI-TOF/TOF tandem MS) methods for obtaining partial sequences of tryptic peptides. MS/MS method was only applied when MALDI-TOF-MS could not provide clear identify of spots.

RESULTS

Cell growth and antibody production To determine the effect of hyperosmotic pressure on rCHO cells (CS13*-1.00) in regard to growth and antibody production, batch cultures with various osmolalities in the range of 300 to 450 mOsm/kg were performed. As shown in figure 1, hyperosmotic pressure depressed cell growth. On the other hand, hyperosmotic pressure significantly enhanced q_{Ab}. The q_{Ab} at 450 mOsm/kg was increased by 139%, compared with that at 300 mOsm/kg.

Proteome profile of rCHO cells Proteome expression profiles of rCHO cells grown in the standard medium (300 mOsm/kg) were shown in figure 2. From the silver-stained 2-D gels, abundant 54 spots were excised and destained. Peptides retrieved from the gel pieces were analyzed by MALDI-TOF-MS. Since the database of CHO cells has not been established, all mass spectra were searched in the database of rodent species or all entries. Among 54 spots, 29 protein spots were identified by MALDI-TOF-MS database search. After MS/MS analysis, additional 12 protein spots could be identified by database search. As a result, the protein expression profile of rCHO cells could be constructed using 41 identified protein spots.

Identification of proteins regulated by hyperosmotic pressure To identify the proteins which are regulated in response to the hyperosmotic pressure, Normalization between 2-D gels with PDQuest™ software was performed based on total density in gel image (Technical note 2566, Bio-rad). This method can be useful if little information is known about sample variation. Compared to standard medium osmolality (300 mOsm/kg), we have found at least 8 differently expressed spots at elevated medium osmolality. Two metabolic enzymes (pyruvate kinase, GAPDH) were found to be up-regulated, suggesting that hyperosmotic pressure can alter metabolic state. The up-regulated activities of these two glycolysis enzymes could activate the generation of metabolic energy, which is consistent with enhanced specific glucose consumption rate at elevated osmolalities. On the other hand, tubulin was found to be down-regulated, suggesting a reduction of the cell division. This result is in agreement with the observation of suppressed cell growth under hyperosmotic pressure.

DISCUSSION

We have undertaken the task of producing a map of proteome of CHO cells. The results found in this study contribute to construction of improved CHO reference map, which can be prerequisite for subsequent comparative proteomic studies of modifications in culture systems. Determining molecular aspects of other stress responses may therefore, provide important useful to further improve animal cell culture performance. Finally, the proteomic effort should continue to improve efficient cell line construction technology, moreover, to develop efficient protein production strategy.

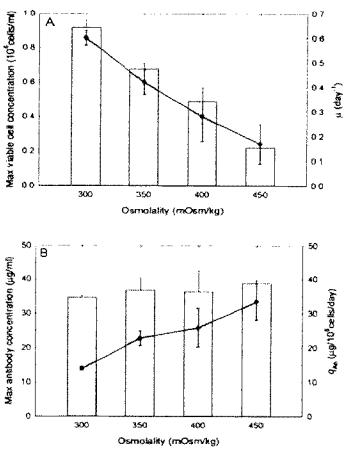

Fig 1. Cell growth (A) and antibody production (B) at various osmolalities

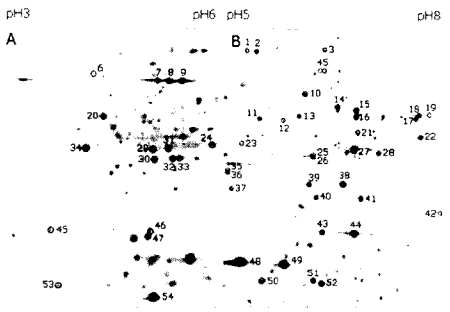

Fig 2. Proteome expression profile of CS13*-1.00 rCHO cells. (300 mOsm/kg). 2mg of proteins were focused on ReadyStrip; (A) pH range 3-6, linear, (B) pH range 5-8, linear. 15% SDS-PAGE gel was used.

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