Efficient production of recombinant IgG by metabolic control and co-expression with GLUT5 in a fructose-based medium

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Abstract A fructose-based cell culture is suitable for the process control of protein production because of slow sugar consumption rate and low lactate accumulation. The fructose transporter, GLUT5, mediates its incorporation into cells and is required for the fructose-based culture. In order to produce efficiently recombinant IgG by metabolic control and co-expression with GLUT5 in a fructose-based medium, an IgG and GLUT5 co-expression vector was constructed and transfected into the human myeloma derived cell line, SC-01MFP, which produced stably recombinant proteins. The cell proliferation in the fructose-based medium was improved by the GLUT5 gene transfection. The recombinant IgG production of the cells cultured in the fructose-based medium exhibited about two-fold increase of that in the glucose-based medium. Flow cytometric analysis indicated that the GLUT5 protein expression level in cell surface was increased in the fructose-based medium. An exogenous but not endogenous GLUT5 transcription activator remarkably

raised IgG productivity in the fructose-based medium when compared to that in the glucose-based medium, suggesting that exogenous GLUT5 expression may be involved in it. The GLUT5 co-expression system may be useful for efficient production of recombinant proteins by the fructose-based cell culture.

Keywords Co-expression · Fructose · GLUT5 · Recombinant IgG · SC-01MFP

Introduction

Glucose is a major component in many mammalian cell culture media, and its consumption results in the accumulation of an undesirable metabolite, lactate, leading to the decrease of cell and product yields due to the acidic shift of pH in the medium. Therefore, sugar metabolic control is one of the approaches for recombinant protein production using mammalian cells. As a substitute for glucose, fructose was known to decrease lactate production (Mochizuki et al. 1991; Petch and Butler 1996), without influencing on the reactivity of monoclonal antibodies (Tachibana et al. 1994; Inoue et al. *in press*). However, not all cell lines can be cultured in a fructose-based medium (Low and Harbour 1985). In our previous study, all-*trans*-retinoic acid (ATRA) was found to improve low proliferation of human hybridomas in the fructose-based medium, and increase the expression of the

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fructose transporter GLUT5 and fructose 1,6-bisphosphatase (FBP; Inoue et al. 2006a, b). GLUT5 mediates exclusively the incorporation of fructose into cells. FBP expression turns the metabolism toward low lactate production. These observations are consistent with the results that have been reported until now. Actually, GLUT5 but not FBP gene transfection improved low proliferation of the hybridomas in a fructose-based medium (Tsukamoto et al. 2010). Thus, we focused on the utilization of GLUT5 for efficient production of recombinant proteins in a fructose-based medium.

We have recently established the human myeloma derived cell line, SC-01MFP to produce recombinant proteins with a human glycosylation profile (Kawahara 2008). These cells produce free lambda light chains of antibodies, and the co-transfection of both light and heavy chain genes to the cells generates intact IgG molecules with two kinds of light chains. In this study, only gamma heavy chain gene was transfected into SC-01MFP cells to simplify the model for recombinant IgG production. We introduce here efficient production of recombinant IgG by metabolic control and co-expression with GLUT5 in a fructose-based medium.

Materials and methods

Cell line and cell culture

The human myeloma derived cell line, SC-01MFP, was established in our laboratory from multiple myeloma RPMI8226 cells (JCRB0034), by selection on the basis of the potential for protein production, cell proliferation and cloning efficiencies (Kawahara 2008). They were maintained in RPMI1640 medium (Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, USA), at 37 °C in humidified 5% CO₂/95% air. In fructose experiments, a glucose-free RPMI1640 medium (Invitrogen, USA) was used, and before experiments, cells were adapted in the medium containing 10%FBS and 2 g/L of fructose for 1 day.

Construction of co-expression vector

An IgG and GLUT5 co-expression vector was constructed from the GLUT5 expression vector, pGLUT5 (Tsukamoto et al. 2010) and the IgG-gamma

chain expression vector, pC5-gamma (Haruta et al. 1997). The *NheI*/*SmaI* fragment containing GLUT5 gene from pGLUT5 was inserted between the *NheI* and *EcoRV* sites of the expression vector pSecTag2C (Invitrogen, USA), and then the *NruI*/*NaeI* fragment containing the IgG-gamma expression construct from pC5-gamma, was inserted into the *NruI* site of the pSecTag2C containing GLUT5 gene as mentioned above. The constructed co-expression vector was referred to as pSec-IgG/GLUT5 (Fig. 1a).

Transfection of expression vector into cells

The pC5-gamma expression vector or the pSec-IgG/GLUT5 co-expression vector was transfected into SC-01MFP cells with Lipofectamine 2000 (Invitrogen, USA) according to a manufacturer's instruction. Transfection was done in 2 mL medium containing 4 µg of DNA, 10 µL of Lipofectamine 2000. After 48 h, the transfected cells were selected and maintained in the 10% FBS-RPMI1640 medium containing 100 µg/mL of G418 for pC5-gamma or 50 µg/mL of Zeocin for pSec-IgG/GLUT5. The resulting two cell populations were referred to as SC-01-IgG or SC-01-IgG/GLUT5, respectively.

Flow cytometric analysis

The SC-01-IgG/GLUT5 (1×10^6 cells) were incubated with rabbit anti-GLUT5 IgG antibodies (SANTA CRUZ, USA) at room temperature for 30 min. After washing in PBS three times, cells were incubated with phycoerythrin (PE)-conjugated mouse anti-rabbit IgG antibodies (SANTA CRUZ, USA) at room temperature for 30 min. After washing in PBS three times, cells were resuspended in PBS. The analyses of PE-labeled cells were performed with a BD FACSVantage SE flow cytometer system (BD, USA).

Activation of GLUT5 expression by transcription activator

Cells (1×10^5 cells/mL) were cultured in the glucose- and fructose-based media supplemented with or without a transcription activator. As the activator of exogenous or endogenous GLUT5 expression, phorbol 12-myristate 13-acetate (PMA) and all-trans retinoic acid (ATRA) was added to the culture medium at final concentrations of 10 or

Fig. 1 GLUT5 co-expression system for efficient production of recombinant IgG. **a** Schematic drawing of the IgG and GLUT5 co-expression vector, pSec-IgG/GLUT5, **b** the principle for increasing IgG production

100 nM, respectively. After 3 days, cell proliferation and recombinant IgG production were measured. Antibody productivity was estimated by dividing total amount of IgG by cumulative viable cell number during the culture.

Measurement of viable cell density and antibody production

Cell number was counted by using a hemacytometer, and viability was determined by the trypan blue dye exclusion method. IgG concentration in cell culture medium was measured by an enzyme-linked immunosorbent assay (ELISA) as described previously (Shoji et al. 1994), using anti-human IgG antibody (AHI1301; Biosource International, Inc., USA) as the first antibody, and anti-human IgG peroxide conjugated

antibody (AHI1304; Biosource International, Inc., USA) as the second antibody.

Statistics

Data are means $\pm$ standard deviations (SD) from triplicate samples. When comparing two data, statistical significance was determined using the Student's *t* test.

Results and discussion

Effect of GLUT5 on cell proliferation and IgG production

To confirm the effectiveness of GLUT5 transfection, SC-01-IgG and SC-01-IgG/GLUT5 cells were cultured

Table 1 Proliferation and IgG production in the fructose-based medium

Cells	Relative cell proliferation	Relative IgG production
SC-01-IgG	0.52 ± 0.01	0.75 ± 0.02
SC-01-IgG/GLUT5	0.86 ± 0.01*	1.84 ± 0.04*

Cells (1×10^5 cells/mL) were cultured in the glucose- and fructose-based media. After 3 days, cell proliferation and recombinant IgG production were compared between both media. Each value in the glucose-based culture is estimated as 1.00. Data represent relative values of means ± SD ($n = 3$). SC-01-IgG vs. SC-01-IgG/GLUT5

* $p < 0.01$ concerns two comparisons in relative cell proliferation and IgG production between SC-01-IgG and SC-01-IgG/GLUT5

in the glucose- and fructose-based media, and then their proliferation and recombinant IgG production were compared between the media. In the SC-01-IgG/GLUT5 cells, the proliferation and IgG production in the fructose-based medium were improved when compared to those in the SC-01-IgG cells (Table 1). In addition, total amount of IgG produced by the SC-01-IgG/GLUT5 cells was increased in the fructose-based medium up to about two-fold of that in the glucose-based medium. This IgG increase was not due to cell proliferation. This suggests that GLUT5 transfection may be effective for recombinant IgG production in the fructose-based medium. On the other hand, the SC-01-IgG cells were not in good conditions in the fructose-based medium, so the SC-01-IgG/GLUT5 cells were only used in the later experiments.

GLUT5 protein expression level in the fructose-based medium

GLUT5 protein expression levels were examined in the glucose- and fructose-based media. Flow cytometry indicated that the peak of GLUT5 in the fructose-based medium shifted to right when compared to that in the glucose-based medium (Fig. 2), suggesting that the GLUT5 protein expression level was increased by fructose. However, the increase rate was low because its expression was restricted to the cell surface and SC-01MFP cells expressed endogenous GLUT5 (Tsukamoto et al. 2010). In this experiment, however, we aimed to confirm the increase of GLUT5 expression in the fructose-based medium, and did not need to discriminate exogenous and endogenous ones.

Fig. 2 Flow cytometric analysis of GLUT5 protein expression at cell surface in the SC-01-IgG/GLUT5 cells

Participation of exogenous GLUT5 in the IgG increase

To examine the participation of exogenous GLUT5 expression in the IgG increase, the SC-01-IgG/GLUT5 cells were treated with PMA that could activate the CMV promoter and increase exogenous expression (Ruybal et al. 2005). Furthermore, to confirm the effect of endogenous GLUT5 on the IgG increase, cells were also done with ATRA that could increase endogenous expression (Inoue et al. 2006a).

Among all cultures tested, cell proliferation was similar (data not shown). As shown in Fig. 3, PMA treatment increased both antibody productivities in the glucose- and fructose-based media. However, this includes direct activation of IgG expression without dependence on exogenous GLUT5 expression, but it also indicates that the co-expression system can work successfully to increase IgG productivity in the fructose-based medium. On the other hand, ATRA

Fig. 3 Activation of exogenous and endogenous GLUT5 expression by PMA and ATRA. Open and solid columns indicate IgG productivities in the glucose- and fructose-based media, respectively. Each column shows the average values and error bars represent the corresponding SD ($n = 3$). * $p < 0.01$ and ** $p < 0.05$ vs. control

treatment exhibited a small increase in the IgG productivity in the fructose-based medium. The ability of PMA and ATRA to activate GLUT5 expression may be different, but these results suggest that exogenous GLUT5 may be at least involved in the IgG increase.

To more increase the IgG productivity, the use of cells that do not express endogenous GLUT5 may be effective. There are some reports that GLUT5 is mainly expressed in the intestine, testis, kidney, skeletal muscle, fat tissue and brain (Kayano et al. 1990; Douard and Ferraris 2008). When the cells without endogenous GLUT5 are used in the transfection experiment of the IgG and GLUT5 co-expression vector, fructose may become a marker for selecting the transfected cells because the untransfected cells can not proliferate in the fructose-based medium. This results in saving the cost for antibiotics that are original selective markers. The selection of high producing clones also leads to more efficient IgG production. Taken together, the GLUT5 co-expression system is useful for efficient production of recombinant IgG using fructose-based cell culture.

Conclusions

We reported here the efficient production of recombinant IgG by the GLUT5 co-expression system in the human SC-01MFP cells. Wlaschin and Hu (2007) reported that CHO cells became proliferative in fructose-based medium by transfection of GLUT5 gene and some clones of them exhibited a significant increase in the final cell concentration in fed-batch culture. Thus our co-expression system may be applicable to CHO cells. The efficient production in SC-01MFP cells is valuable for functional analysis of recombinant proteins with a profile of human glycosylation profile.

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