BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

Effect of sodium butyrate on the assembly, charge variants, and galactosylation of antibody produced in recombinant Chinese hamster ovary cells

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Abstract Sodium butyrate (NaBu) is known to increase the specific productivity of recombinant Chinese hamster ovary (rCHO) cells. To understand the effects of NaBu on the product quality, rCHO cells producing monoclonal antibody (Mab) were cultivated at various concentrations of NaBu (0 to 4 mM). NaBu increased correctly assembled Mab. In the absence of NaBu, the proportions of intact Mab (2H2L) and heavy chain dimer (2H) were 81 and 15 %. At 1 mM NaBu, the proportion of 2H2L increased to 93 %, whereas the proportion of 2H decreased to 2 %. No further increase in the proportion of 2H2L was obtained at a higher NaBu concentration. NaBu also affected the charge heterogeneity of Mab, which may affect the efficacy of Mab. The basic charge variants of Mabs increased with an increase in the NaBu concentration. In addition, NaBu affected the galactosylation of Mab negatively. Overall, the data obtained here show that NaBu used in rCHO cell cultures for improved Mab production affects certain quality aspects of Mab, in this case, the charge heterogeneity and galactosylation.

Keywords Antibody · Assembly · Charge heterogeneity · CHO cells · Galactosylation · Sodium butyrate

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Introduction

Therapeutic monoclonal antibodies (Mabs) of the immunoglobulin G (IgG) class that are produced predominantly from Chinese hamster ovary (CHO) cell cultures have proven to be invaluable pharmaceuticals for the treatment of various diseases (Brekke and Sandlie 2003).

IgG Mabs are glycoproteins consisting of two identical heavy chains (HCs) and two identical light chains (LCs) (Jefferis 2009). Product heterogeneity, especially in terms of the size, charge, and glycosylation (Hossler et al. 2009; Liu et al. 2008; Vlasak and Ionescu 2008), is common to Mabs and is typically introduced either upstream during expression or downstream during manufacturing. As the therapeutic efficacy of Mab varies depending on quality aspects such as its assembly, charge modification, and glycosylation (Boswell et al. 2010; Khawli et al. 2010; Pan et al. 2009), critical quality attributes as well as productivity should be considered thoroughly during the process development of Mabs.

Sodium butyrate (NaBu), a short-chain fatty acid, has been widely used to increase foreign protein productivity (q_p) in recombinant CHO (rCHO) cells producing erythropoietin (Yoon et al. 2004), tissue plasminogen activator (Hendrick et al. 2001), thrombopoietin (Sung et al. 2004), follicle-stimulating hormone (Chotigeat et al. 1994), and Mab (Hong et al. 2011; Jiang and Sharfstein 2008; Mimura et al. 2001). NaBu is also known to induce apoptosis in CHO cell cultures (Lee and Lee 2012; Sung and Lee 2005). Recently, it was reported that the pro-apoptotic effects of NaBu are associated with oxidative stress (Chang et al. 2013; Louis et al. 2004; Vanhoutvin et al. 2009). In rCHO cells producing factor VIII, a NaBu treatment induced intracellular reactive oxygen species (ROS) and apoptosis, whereas an antioxidant treatment reduced both (Malhotra et al. 2008). These results suggest that CHO cells exposed to NaBu undergo oxidative

stress. Likewise, a NaBu treatment may also affect the quality of Mabs produced in rCHO cells.

Although NaBu has been widely used in rCHO cell cultures for improved Mab production, studies of its effect on certain aspects of the quality of Mab remain not fully substantiated. In this context, we investigated the effect of NaBu on the modification of Mabs produced in rCHO cells (CS13-1.0) with regard to its assembly, charge heterogeneity, and glycosylation characteristics.

Materials and methods

Cell line and culture medium

rCHO cells producing an antibody against the S surface antigen of the hepatitis B virus (CS13-1.0) were used. As described previously (Kim et al. 1998), they were established through the co-transfection of vectors containing dihydrofolate reductase (dhfr) and antibody genes into DG44 with a subsequent dhfr/methotrexate (MTX)-mediated gene amplification step up to 1 μM MTX (Sigma-Aldrich, St. Louis, MO). Cells were adapted for growth in a serum-free suspension culture in 125-mL Erlenmeyer flasks (Corning, Corning, NY) containing 40 mL of HyClone SFM4CHO^{TM} (Hyclone, Logan, UT) supplemented with 4 mM glutamine and 1 μM MTX in a Climo-Shaker CO$_2$ incubator (ISF1-X, Adolf Kuhner AG, Birsfelden, Switzerland) set at 110 rpm, 85 % humidity, and 37 °C.

Cell culture, viable cell concentration, and antibody assay

Exponentially growing cells were inoculated at a concentration of 5.0×10^5 cells/mL in Erlenmeyer flasks containing 40 mL of culture media with NaBu concentrations ranging from 0 to 4 mM, after which the flasks were incubated in a Climo-Shaker CO$_2$ incubator set at 110 rpm, 85 % humidity, and 37 °C. Approximately 1 mL of culture media was taken daily from the flask for analysis. The cell concentration was then estimated using a hemacytometer, and viable cells were distinguished from dead cells using the trypan blue dye exclusion method. Cell lysates and culture supernatants were aliquoted and maintained in a frozen state at −70 °C for later analysis. The secreted antibody concentration was quantified using an enzyme-linked immunosorbent assay (ELISA), as described previously (Kim et al. 1998). Experiments were repeated two separate times.

Western blotting analysis

Western blot analyses were performed as described previously (Hwang and Lee 2008). The antibodies used for immunoblotting were the anti-β-actin mouse monoclonal antibody (Clone

AC-74, Sigma), anti-Grp78/Bip rabbit polyclonal antibody (SPA-826, Stressgen, York, UK), peroxidase-conjugated anti-rabbit IgG goat polyclonal antibody (7074, Cell Signaling, Hitchin, UK), peroxidase-conjugated anti-mouse IgG goat polyclonal antibody (Upstate Biotechnology, Lake Placid, NY), anti-human IgG (Fc specific)-peroxidase conjugate (A0170, Sigma), and the anti-human kappa LC-peroxidase conjugate (A7164, Sigma). Bands were then visualized by the ECL Western blot analysis system (GE Healthcare, Buckinghamshire, UK).

Purification of recombinant antibody

Culture medium was taken from the flasks. After centrifugation, the secreted antibody in the culture supernatants was purified by protein A affinity chromatography (recombinant protein A agarose, Pierce, Rockford, IL) according to the manufacturer's instructions.

Capillary electrophoresis–sodium dodecyl sulfate (CE-SDS) analysis

A non-reduced CE-SDS analysis was performed on a ProteomeLab™ PA 800 plus pharmaceutical analysis system with 32 Karat software (Beckman Coulter, Fullerton, CA) according to the manufacturer's instructions. A bare-fused silica capillary (50 μm ID×30.2 cm) and an IgG purity/heterogeneity assay kit were obtained from Beckman Coulter.

Capillary isoelectric focusing (cIEF) analysis

A cIEF analysis was also performed on a PA800 plus pharmaceutical analysis system with 32 Karat software according to the manufacturer's instructions. The cIEF reagents, neutrally coated capillary, gel, and cIEF peptide markers were obtained from Beckman Coulter. The sample buffer was prepared by mixing pI standard markers with a desalted protein sample.

IgG oxidation via a hydrogen peroxide (H$_2$O$_2$) treatment

H$_2$O$_2$ experiments were performed with Mabs produced in the absence of NaBu. Purified Mabs were diluted to 5 mg/mL in a 10 mM Na-phosphate buffer (pH 7.0). After H$_2$O$_2$ (Sigma) was added to the Mab solution at a final concentration of 0.3 %, the Mab solution was incubated at room temperature (25 °C) for the indicated times (0, 1, 3, and 6 h). Then, the H$_2$O$_2$ in the Mab solution was removed by a buffer exchange method (1,000 volumes of precooled 10 mM Na-phosphate buffer (pH 7.0)), and the Mabs were reconcentrated at a concentration of 5 mg/mL. The Mabs were then analyzed by cIEF.

CE-based carbohydrate analysis

Glycans were released from reduced IgG using the PNGase F enzyme. The samples were incubated at 37 °C overnight and isolated. 1-Aminopyrene-3,6,8-trisulfonic acid (APTS) labeling of samples was undertaken using the ProteomeLab™ carbohydrate labeling and analysis kit from Beckman Coulter. A CE-based carbohydrate analysis was performed on a ProteomeLab™ PA 800 System by means of laser-induced fluorescence (LIF) detection with an excitation wavelength of 488 nm and an emission band-pass filter of 520±10 nm. An N-CHO-coated capillary 50 μm ID×50.2 cm in size was used. Sample preparation, separation, and analysis were done according to the manufacturer's instructions.

Results

Effect of NaBu on cell growth and antibody production

To investigate the effects of NaBu on cell growth and antibody production, cells were cultivated in SFM4CHO containing various concentrations of NaBu. Cell culture was performed two separate times.

Figure 1 shows the cell growth, cell viability, and antibody production profiles during the cultures. NaBu inhibited cell growth and decreased cell viability in a dose-dependent manner. The maximum viable cell concentration and specific growth rate (μ) in the absence of NaBu ($3.49\pm0.11\times10^6$ cells/mL and 0.51 ± 0.07 day^{-1}) decreased to $0.73\pm0.02\times10^6$ cells/mL and 0.22 ± 0.01 day^{-1} at 1 mM NaBu, respectively. The cells could not grow much, and cell viability decreased rapidly at more than 1 mM NaBu (Fig. 1a, b).

Despite the inhibited cell growth by NaBu, the highest antibody concentration (283.6 ± 7.6 μg/mL) was achieved at 0.2 mM NaBu, suggesting that NaBu enhanced the specific antibody productivity (q_{Mab}) (Fig. 1c). Additionally, q_{Mab} in the absence of NaBu (13.5 ± 1.0 pg/cell/day) increased to 72.2 ± 2.2 pg/cell/day with 4 mM NaBu. Thus, a NaBu addition decreased μ and increased q_{Mab} in a dose-dependent manner, as summarized in Table 1.

Effect of NaBu on antibody assembly

To evaluate the effect of NaBu on the antibody assembly characteristics, a non-reduced CE-SDS analysis was performed with Mabs purified by protein A resin on day 5 (NaBu 0, 0.01, 0.05, 0.2, and 1 mM), on day 4 (NaBu 2 mM), and on day 3 (NaBu 4 mM) of the cultures shown in Fig. 1. The protein A resin binds with the Fc region of IgG; therefore, purified antibodies do not contain a free LC monomer (1 L) or a LC dimer (2 L).
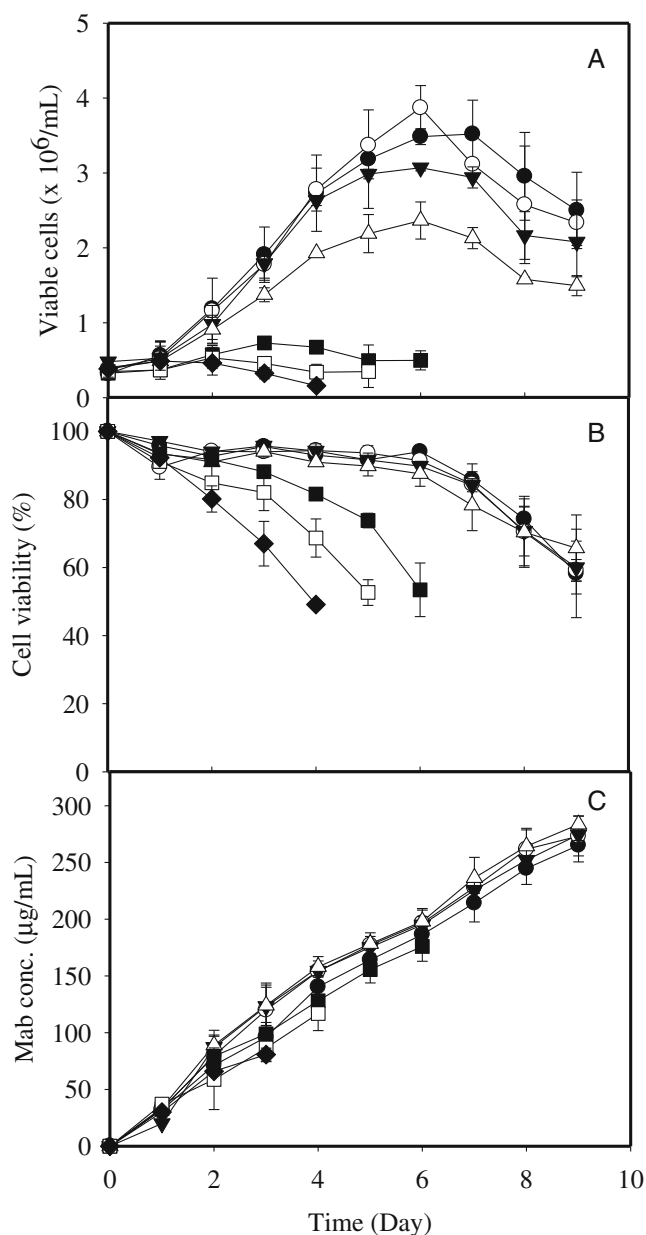

Fig 1 Profiles of the cell growth, cell viability, and Mab production at various concentrations of NaBu (●: 0 mM NaBu, ○: 0.01 mM NaBu, ▼: 0.05 mM NaBu, △: 0.2 mM NaBu, ■: 1 mM NaBu, □: 2 mM NaBu, ♦: 4 mM NaBu). **a** Viable cell concentration. **b** Cell viability. **c** Mab concentration. Cell cultures were performed two separate times. The *error bars* represent standard deviations calculated from the data obtained in two independent experiments

Figure 2 shows profiles of the Mab size variants as analyzed by non-reduced CE-SDS. In all samples, three major peaks were clearly separated and identified in a comparison with the bands in the non-reduced Western blotting analysis of purified IgG against HC as well as LC samples (data not shown). The main peak corresponds to an intact IgG (2H2L) tetramer, and the adjacent peak represents a species lacking one LC (2H1L). The third peak from the right side is a HC dimer (2H). A 10-kDa internal standard was loaded with the

Table 1 Effect of NaBu on μ, q_{Mab}, and maximum antibody concentration

NaBu concentration (mM)	μ (day^{-1})	q_{Mab} (pg/cell/day)	Max. antibody concentration (μg/mL)
0	0.509±0.065	13.50±0.97	265.2±14.6
0.01	0.478±0.050	14.38±1.44	273.3±17.5
0.05	0.437±0.039	15.53±0.85	274.6±5.2
0.2	0.352±0.009	21.75±3.95	283.6±±7.6
1	0.221±0.007	58.05±4.32	176.0±13.0
2	0.109±0.006	68.35±3.57	116.8±±15.0
4	0.001±0.089	72.22±2.21	80.7±5.7

Values are means±standard deviations of two independent experiments
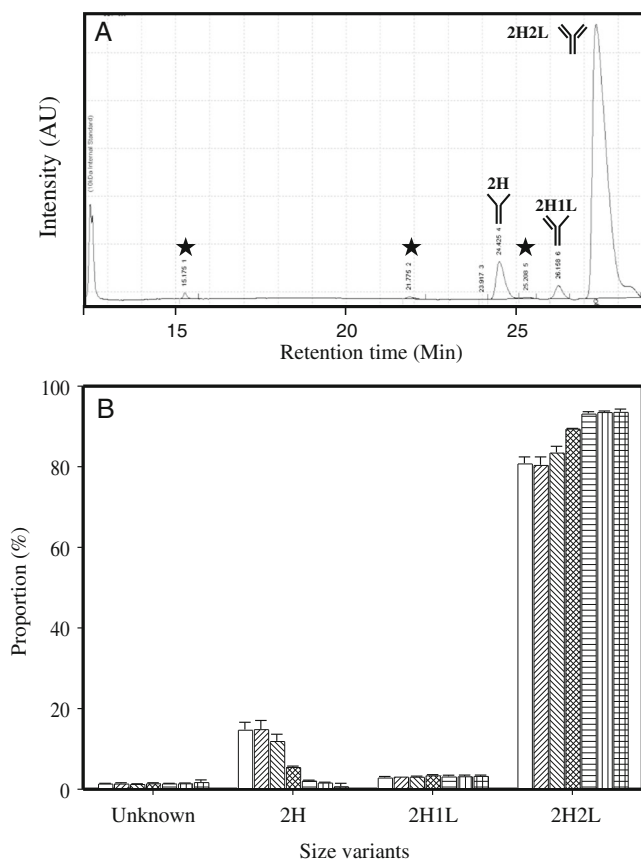

Fig. 2 Profiles of Mab size variants analyzed by non-reduced CE-SDS. **a** A typical non-reduced CE-SDS separation of Mab. Unknown: the peaks of uncharacterized variants indicated with *stars*. **b** A proportion of the size variants calculated from the area of the electropherogram peak. (□: 0 mM NaBu, ▨: 0.01 mM NaBu, ▨: 0.05 mM NaBu, ▨: 0.2 mM NaBu, ▤: 1 mM NaBu, ▥: 2 mM NaBu, ▦: 4 mM NaBu). The *error bars* represent standard deviations calculated from the data obtained in two independent experiments. Proportions of 2H and 2H2L on each concentration of NaBu were regressed by non-linear curved fitting. The model equation was analyzed to be reliable (*p* value <0.05, R^2>95 %), indicating that the decrease of 2H as well as the increase of 2H2L caused by NaBu was statistically reliable

samples as a control (Fig. 2a). Based on the area of the electropherogram peaks, the proportions of the size variants were determined and plotted (Fig. 2b).

In the absence of NaBu, the proportion of intact IgG was 81 %, while the proportion of 2H was 15 %. Interestingly, with the addition of increasing concentrations of NaBu up to 1 mM, the proportion of intact IgG increased significantly with a concomitant decrease in the proportion of 2H. At 1 mM NaBu, the proportions of 2H2L and 2H were 93 and 2 %, respectively. Further increases in the NaBu concentrations of up to 4 mM did not increase the portion of 2H2L. The proportion of 2H1L and unknown peaks did not change significantly regardless of the NaBu concentration.

Given that 1 L and 2 L in the culture supernatants were not recovered by protein A affinity chromatography, non-reduced culture supernatants, sampled on day 3, were loaded onto the SDS-GEL and blotted against LC as well as HC in order to identify any antibody size variants in the culture supernatants.

Figure 3 shows Western blots of the non-reduced culture supernatants. With anti-HC antibody, two major bands, 150 and 100 kDa, were detected (Fig. 3a). The intensity of the 100-kDa band, which corresponds to 2H, decreased with an increase in the concentration of NaBu, in accordance with the results shown in Fig. 2b. The intensity of the 150-kDa band, which corresponds to 2H2L, did not change significantly with the NaBu concentration, which was also confirmed with the anti-LC antibody (Fig. 3b). With the anti-LC antibody, three major bands (150, 50, and 25 kDa) were detected. The 50- and 100-kDa bands correspond to L and 2 L, respectively. In contrast with H2, the intensity levels of 1 L and 2 L increased with an increase in the concentration of NaBu.

Intracellular levels of antibody and Grp78/Bip

To investigate whether an addition of NaBu resulted in changes in intracellular antibodies or Grp78/Bip, Western blotting analyses against HC and LC and Grp78/Bip were conducted with non-reduced cell lysates sampled on day 3 of the cultures shown in Fig. 1.

Figure 4 shows Western blots of the non-reduced cell lysates. With anti-HC and anti-LC antibodies, several bands were detected (Fig. 4a, b). Based on the molecular weights of HC and LC, each band was identified as the size variant of the antibody. Overall, the intracellular levels of all types of antibody size variants increased with an increase in the concentration of NaBu up to 1 mM, after which they became saturated.

The level of Grp78/Bip also increased as the concentration of NaBu increased (Fig. 4c). Grp78/Bip is one of the major chaperones interacting with polypeptide to fold correctly in the endoplasmic reticulum (ER) (Lin et al. 1993; Melnick et al. 1994). Thus, the accumulation of these polypeptides in

Fig. 3 Western blot analysis of non-reduced culture supernatants. **a** Western blotting against HC. **b** Western blotting against LC
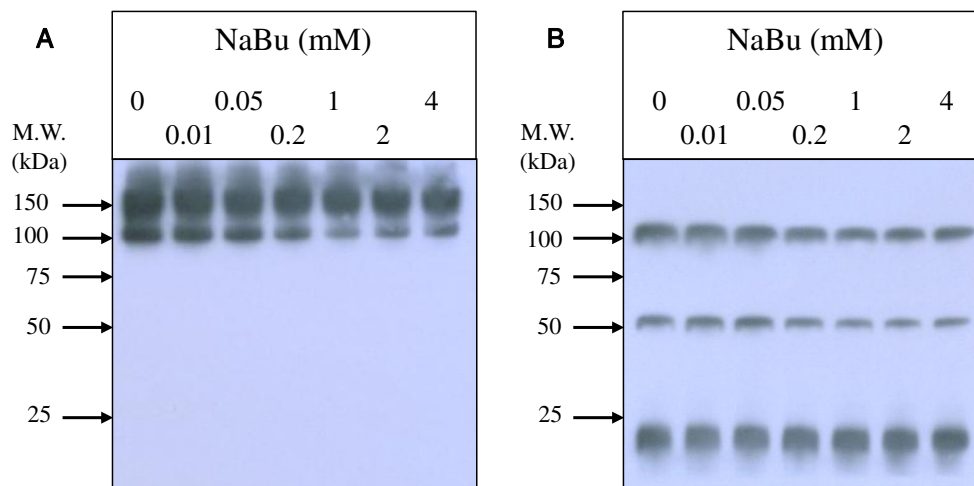
the ER at high NaBu concentrations may induce Grp78/Bip expression.

Effect of NaBu on the charge heterogeneity of the antibody

To evaluate the effect of NaBu on the charge heterogeneity of the antibody, cIEF was performed with Mabs purified by protein A resin at the time points indicated in Fig. 2.

Figure 5 shows the cIEF profiles of Mabs. Mabs produced in the absence of NaBu were separated into six peaks of charge variants according to their own pI (B3, B2, B1, M,
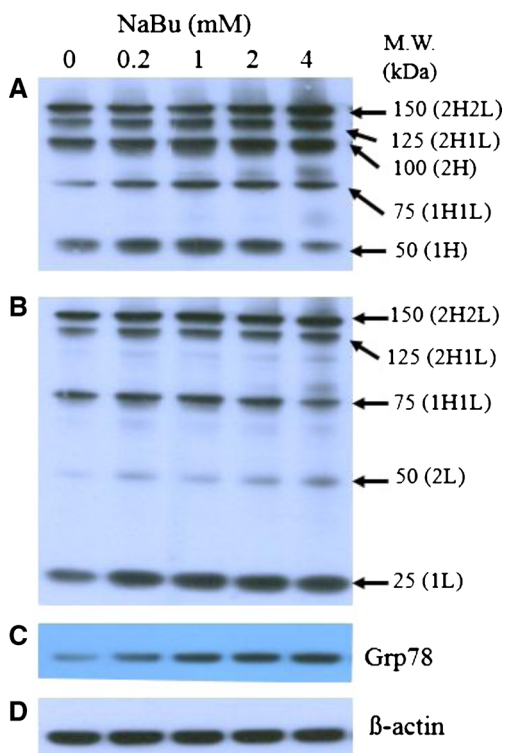

Fig. 4 Western blot analysis of non-reduced cell lysates. **a** Western blotting against HC. **b** Western blotting against LC. **c** Western blotting against Grp78/Bip. **d** Western blotting against β-actin used as a control

A1, and A2). In addition, B1, M, and A1 peaks were shown to form a cluster.

By comparing the peaks of the cIEF profiles with the proportions of the size variants in Fig. 2b, the B3 peak can be inferred as belonging to 2H. With an increase in the concentration of NaBu, the B3 peak, like the 2H size variant shown in Fig. 2b, decreased, supporting the contention that the B3 peak belongs to 2H. Likewise, the cluster of the B1, M, and A1 peaks can be inferred as belonging to 2H2L. This also indicates that 2H2L, as noted in a single peak in Fig. 2b, consists of the charge variants B1, M, and A1.

With an increase in the concentration of NaBu, the M peak (pI 7.68) and A1 peak (pI 7.63) decreased, while the B1 peak (pI 7.72) increased. In addition, more basic peaks such as B1′ (pI 7.77) and B1″ (pI 7.81) appeared and increased at higher NaBu concentrations. At 4 mM NaBu, the B1″ peak had the largest portion of cIEF peaks, indicating that NaBu addition to culture media increased the basic charge variants of Mab produced in CHO cells.

To determine whether oxidative stress induced by NaBu addition increased the basic charge variants of the Mabs, the Mabs produced in the absence of NaBu were treated with representative pro-oxidant, H_2O_2 (0.3 %v/v), for 6 h at room temperature.

Figure 6 shows the cIEF profiles of Mabs treated with H_2O_2. The levels of B3, B2, A1, and A2 were not affected by the 6-h H_2O_2 treatment. However, the H_2O_2 treatment decreased the M peak (main peak), while increasing the B1 peak (basic charge variant). This result suggests that oxidative stress induced by an addition of NaBu is responsible in part for the increased basic charge variants shown in Fig. 5.

Effect of NaBu on antibody galactosylation

To evaluate the effect of NaBu on the antibody glycan profiles, the N-linked glycans from Mabs purified by protein A resin on day 5 (NaBu 0, 0.2, and 1 mM), on day 4 (NaBu
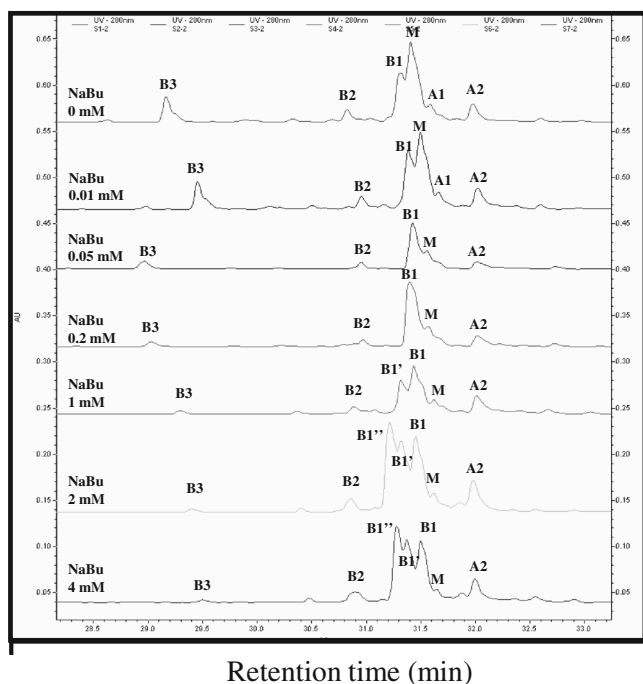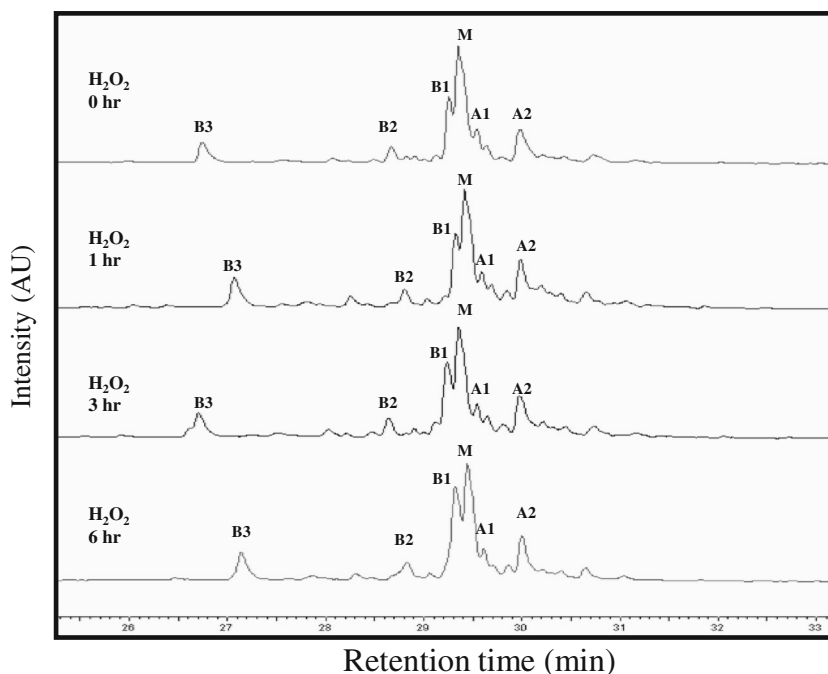
Fig. 5 cIEF profiles of Mabs produced at various concentrations of NaBu. *M* main peak, *B* basic peak, *A* acidic peak

2 mM), and on day 3 (NaBu 4 mM) of the cultures shown in Fig. 1 were released using PNGase F enzyme.

Figure 7 shows a typical electropherogram profile of the APTS-labeled glycans separated using CE-LIF. The proportions of glycans that were determined based on the area of the chromatogram peak are also plotted in Fig. 7.

G0, G0F, G1F(1,6), G1F′(1,3), and G2F, which are typical for Mabs produced in rCHO cells, were identified by comparisons with glucose ladder standards. In the absence of NaBu, the proportions of G0+G0F, G1F+G1F′, and G2F were 66.7, 25.4, and 3.2 %, respectively. With an increase in the concentration of NaBu up to 1 mM, the proportion of G0+G0F increased with a concomitant decrease in the proportions of G1F+G1F′ and G2F. However, further increases in the NaBu concentration up to 4 mM did not change their proportions significantly. At 1 mM NaBu, the proportions of G0+G0F, G1F+G1F′, and G2F were 75.8, 15.2, and 1.4 %, respectively. This result indicates that an addition of NaBu has a negative effect on antibody galactosylation.

Discussion

The addition of NaBu to culture media significantly enhanced the q_p values of many rCHO cell lines (Kim and Lee 2002; Rodriguez et al. 2005; Sung and Lee 2005; Lee et al. 2014). The q_{Mab} of the rCHO cells (CS13-1.0) used in this study was also enhanced in the presence of NaBu. Although its beneficial effect on q_p is well known, its effect on the quality of recombinant protein is relatively undiscovered. Thus, we investigated the effect of NaBu on the quality of Mabs produced in rCHO cells (CS13-1.0) with regard to assembly, charge heterogeneity, and galactosylation.

A NaBu addition was found to increase properly assembled antibody, which could be another beneficial effect of adding NaBu during the production of Mab in rCHO cell cultures.
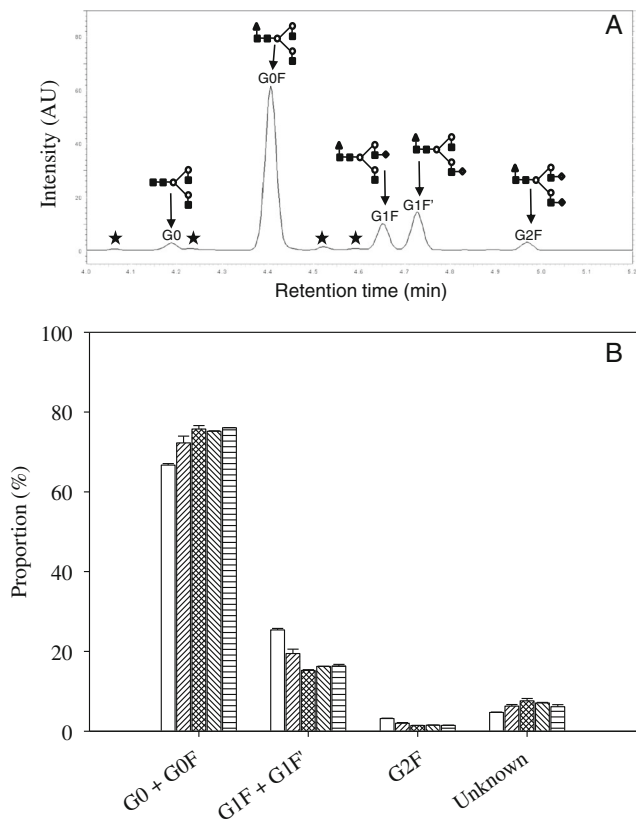
Fig. 6 cIEF profiles of Mabs treated with H_2O_2. *M* main peak, *B* basic peak, *A* acidic peak. Mabs produced on day 5 in the absence of NaBu were purified and then treated with H_2O_2 (0.3 %*v/v*) for 0, 1, 3, and 6 h

Fig. 7 Electropherogram profiles of the APTS-labeled glycans separated using CE-LIF. **a** Electropherogram profile of APTS-labeled glycans in the absence of NaBu. Unknown: the peak of uncharacterized glycan indicated with a *star*. **b** The proportions of glycans calculated from a glycan analysis (□: 0 mM NaBu, ▨: 0.2 mM NaBu, ▓: 1 mM NaBu, ▧: 2 mM NaBu, ≡: 4 mM NaBu). *G0* agalactosylated glycan without fucose, *G0F* agalactosylated glycan with fucose, *G1F* monogalactosylated (α 1–6) glycan with fucose, *G1F′* monogalactosylated (α 1–3) glycan with fucose, *G2F* digalactosylated glycan with fucose. The *error bars* represent standard deviations calculated from the data obtained in two independent experiments

When CS13-1.0 cells were cultivated in the absence of NaBu, the proportion of intact IgG, 2H2L, was 81 % while that of 2H was 15 %. Interestingly, an addition of 1 mM NaBu to the culture medium significantly increased the proportion of 2H2L (93 %) while decreasing that of 2H (2 %). IgG Mab consists of two HCs and two LCs. The two HCs are linked to each other, and each HC is linked to a LC by a disulfide bond. Thus, the increased proportion of 2H2L by NaBu suggests that an addition of NaBu is beneficial to form a disulfide bond in the inter-molecular linkage between HC and LC.

In rCHO cells producing Mab, two HCs and two LCs are folded and assembled in the ER (Liu et al. 2008; Vlasak and Ionescu 2008). The LC polypeptide is known to be synthesized 10–15 % faster than the HC polypeptide (Bergman et al. 1981; Leitzgen et al. 1997; Reddy et al. 1996; Shapiro et al. 1966). In addition, it is easily folded with transient association with Grp78/Bip and can be secreted as a monomer or a dimer. In contrast, the HC polypeptide interacts extensively with Grp78/Bip. Increased HC expression in the absence of the LC polypeptide results in the accumulation of unfolded HC polypeptide in the ER, which induces the expression of chaperones such as Grp78/Bip and PDI.

In the presence of NaBu, the expression levels of LC and HC increased in CS13-1.0 cells (Figs. 3 and 4). Likewise, the expression level of Grp78/Bip increased most likely in order to process the increased LC and HC polypeptides. Due to the shorter synthesis rate of LC polypeptide, the expression level of the LC polypeptide was much higher than that of the HC polypeptide in the ER. Thereby, the HC polypeptide was expected to have a greater potential to assemble with the LC polypeptide, far from being secreted as a type of 2H (Schlatter et al. 2005). It is also likely that the increased level of the chaperones contributed to a proper assembly of the antibody in the ER.

A NaBu addition was found to increase basic charge variants of Mabs produced in CS13-1.0 cells. With an increase in the concentration of NaBu, even the main peak of the charge variants was shifted to the basic charge variants. A variety of antibody modifications are known to influence the charge heterogeneity of Mabs (Liu et al. 2008; Vlasak and Ionescu 2008). In many cases, the basic charge variants in Mab are caused by the oxidation of labile amino acid residues (methionine, cysteine, lysine, histidine, and tryptophan), as induced by temperature and oxidative chemicals during the cell culture and purification processes as well as in storage (Chumsae et al. 2007; Harris et al. 2001; Harris et al. 2004; Kaschak et al. 2011; Zhang et al. 2011). Thus, the increased basic charge variants of the Mabs by NaBu may originate in part from intracellular oxidative stress such as ROS. In fact, several studies have reported that a NaBu addition induced intracellular ROS and oxidative damages (Chang et al. 2013; Louis et al. 2004; Malhotra et al. 2008; Vanhoutvin et al. 2009). When purified Mab was treated with H_2O_2, a well-known pro-oxidant, the basic charge variants increased with the incubation time. This result indicates that the increased basic charge variants in the Mabs produced in rCHO cells in the presence of NaBu were in part due to NaBu-induced ROS in rCHO cells. If these basic charge modifications occur in the active sites of Mab, such as in the epitope or effector region, the antigen binding efficiency or therapeutic efficacy can be greatly affected. Thus, Mabs produced in the cell culture process with NaBu need to be characterized in depth.

An addition of NaBu was found to have a negative effect on the galactosylation of Mabs produced in CS13-1.0 cells, in contrast with earlier findings (Mimura et al. 2001). Negative effects of a NaBu addition on galactosylation in Mabs, as observed in this study, can be a concern because the galactosylation of Fc-glycan modulates the effector functions and pharmacokinetic properties. Previously, it was also reported that a NaBu addition reduced the sialylation of glycoproteins such as GP1-IgG, interferon-beta, and thrombopoietin

produced in rCHO cells (Santell et al. 1999; Rodriguez et al. 2005; Sung et al. 2004).

In conclusion, a NaBu addition to a culture medium increased the q_{Mab} of rCHO cells while also affecting the quality of Mab. It increased correct antibody assembly, resulting in an improved proportion of intact Mab. However, it also increased the basic charge variants of Mabs while negatively affecting the galactosylation of Mabs. Thus, the use of NaBu in rCHO cell cultures for improved Mab production requires an in-depth characterization of Mabs.

References

Bergman LW, Harris E, Kuehl WM (1981) Glycosylation causes an apparent block in translation of immunoglobulin heavy chain. J Biol Chem 256(2):701–716

Boswell CA, Tesar DB, Mukhyala K, Theil FP, Fielder PJ, Khawli LA (2010) Effects of charge on antibody tissue distribution and pharmacokinetics. Bioconjug Chem 21(12):2153–2163

Brekke OH, Sandlie I (2003) Therapeutic antibodies for human diseases at the dawn of the twenty-first century. Nat Rev Drug Discov 2:52–62

Chang MC, Tsai YL, Chen YW, Chan CP, Huang CF, Lan WC, Lin CC, Lan WH, Jeng JH (2013) Butyrate induces reactive oxygen species production and affects cell cycle progression in human gingival fibroblasts. J Periodontal Res 48(1):66–73

Chotigeat W, Watanapokasin Y, Mahler S, Gray PP (1994) Role of environmental conditions on the expression levels, glycoform pattern and levels of sialyltransferase for hFSH produced by recombinant CHO cells. Cytotechnology 15(1–3):217–221

Chumsae C, Gaza-Bulseco G, Sun J, Liu H (2007) Comparison of methionine oxidation in thermal stability and chemically stressed samples of a fully human monoclonal antibody. J Chromatogr B Analyt Technol Biomed Life Sci 850(1–2):285–294

Harris RJ, Kabakoff B, Macchi FD, Shen FJ, Kwong M, Andya JD, Shire SJ, Bjork N, Totpal K, Chen AB (2001) Identification of multiple sources of charge heterogeneity in a recombinant antibody. J Chromatogr B 752(2):233–245

Harris RJ, Shire SJ, Winter C (2004) Commercial manufacturing scale formulation and analytical characterization of therapeutic recombinant antibodies. Drug Dev Res 61(3):137–154

Hendrick V, Winnepenninckx P, Abdelkafi C, Vandeputte O, Cherlet M, Marique T, Renemann G, Loa A, Kretzmer G, Werenne J (2001) Increased productivity of recombinant tissular plasminogen activator (t-PA) by butyrate and shift of temperature: a cell cycle phases analysis. Cytotechnology 36(1–3):71–83

Hong JK, Lee GM, Yoon SK (2011) Growth factor withdrawal in combination with sodium butyrate addition extends culture longevity and enhances antibody production in CHO cells. J Biotechnol 155(2):225–231

Hossler P, Khattak SF, Li ZJ (2009) Optimal and consistent protein glycosylation in mammalian cell culture. Glycobiology 19(9):936–949

Hwang SO, Lee GM (2008) Nutrient deprivation induces autophagy as well as apoptosis in Chinese hamster ovary cell culture. Biotechnol Bioeng 99(3):678–685

Jefferis R (2009) Glycosylation as a strategy to improve antibody-based therapeutics. Nat Rev Drug Discov 8:226–234

Jiang Z, Sharfstein ST (2008) Sodium butyrate stimulates monoclonal antibody over-expression in CHO cells by improving gene accessibility. Biotechnol Bioeng 100(1):189–194

Kaschak T, Boyd D, Lu F, Derfus G, Kluck B, Nogal B, Emery C, Summers C, Zheng K, Bayer R, Amanullah A, Yan BX (2011) Characterization of the basic charge variants of a human IgG1: effect of copper concentration in cell culture media. Mabs-Austin 3(6):577–583

Khawli LA, Goswami S, Hutchinson R, Kwong ZW, Yang JH, Wang XD, Yao ZL, Sreedhara A, Cano T, Tesar D, Nijem I, Allison DE, Wong PY, Kao YH, Quan C, Joshi A, Harris RJ, Motchnik P (2010) Charge variants in IgG1 Isolation, characterization, in vitro binding properties and pharmacokinetics in rats. Mabs-Austin 2(6):613–624

Kim NS, Lee GM (2002) Inhibition of sodium butyrate-induced apoptosis in recombinant Chinese hamster ovary cells by constitutively expressing antisense RNA of caspase-3. Biotechnol Bioeng 78(2):217–228

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(1):73–84

Lee JS, Lee GM (2012) Effect of sodium butyrate on autophagy and apoptosis in Chinese hamster ovary cells. Biotechnol Prog 28:349–357

Lee SM, Kim YG, Lee EG, Lee GM (2014) Digital mRNA profiling of N-glycosylation gene expression in recombinant Chinese hamster ovary cells treated with sodium butyrate. J Biotechnol 171:56–60

Leitzgen K, Knittler MR, Haas IG (1997) Assembly of immunoglobulin light chains as a prerequisite for secretion. A model for oligomerization-dependent subunit folding. J Biol Chem 272(5):3117–3123

Lin HY, Massowelch P, Di YP, Cai JW, Shen JW, Subjeck JR (1993) The 170-kDa glucose-regulated stress protein is an endoplasmic reticulum protein that binds immunoglobulin. Mol Biol Cell 4(11):1109–1119

Liu H, Gaza-Bulseco G, Faldu D, Chumsae C, Sun J (2008) Heterogeneity of monoclonal antibodies. J Pharm Sci 97(7):2426–2447

Louis M, Rosato RR, Brault L, Osbild S, Battaglia E, Yang XH, Grant S, Bagrel D (2004) The histone deacetylase inhibitor sodium butyrate induces breast cancer cell apoptosis through diverse cytotoxic actions including glutathione depletion and oxidative stress. Int J Oncol 25(6):1701–1711

Malhotra JD, Miao H, Zhang K, Wolfson A, Pennathur S, Pipe SW, Kaufman RJ (2008) Antioxidants reduce endoplasmic reticulum stress and improve protein secretion. Proc Natl Acad Sci U S A 105(47):18525–18530

Melnick J, Dul JL, Argon Y (1994) Sequential interaction of the chaperones BiP and GRP94 with immunoglobulin chains in the endoplasmic reticulum. Nature 370(6488):373–375

Mimura Y, Lund J, Church S, Dong S, Li J, Goodall M, Jefferis R (2001) Butyrate increases production of human chimeric IgG in CHO-K1 cells whilst maintaining function and glycoform profile. J Immunol Methods 247(1–2):205–216

Pan H, Chen K, Chu L, Kinderman F, Apostol I, Huang G (2009) Methionine oxidation in human IgG2 Fc decreases binding affinities to protein A and FcRn. Protein Sci 18(2):424–433

Reddy P, Sparvoli A, Fagioli C, Fassina G, Sitia R (1996) Formation of reversible disulfide bonds with the protein matrix of the endoplasmic reticulum correlates with the retention of unassembled Ig light chains. EMBO J 15(9):2077–2085

Rodriguez J, Spearman M, Huzel N, Butler M (2005) Enhanced production of monomeric interferon-beta by CHO cells through the control of culture conditions. Biotechnol Prog 21(1):22–30

Santell L, Ryll T, Etcheverry T, Santoris M, Dutina G, Wang A, Gunson J, Warner TG (1999) Aberrant metabolic sialylation of recombinant proteins expressed in Chinese hamster ovary cells in high productivity cultures. Biochem Biophys Res Commun 258(1):132–137

Schlatter S, Stansfield SH, Dinnis DM, Racher AJ, Birch JR, James DC (2005) On the optimal ratio of heavy to light chain genes for efficient recombinant antibody production by CHO cells. Biotechnol Prog 21(1):122–133

Shapiro AL, Scharff MD, Maizel JV, Uhr JW (1966) Synthesis of excess light chains of gamma globulin by rabbit lymph node cells. Nature 211(5046):243–245

Sung YH, Lee GM (2005) Enhanced human thrombopoietin production by sodium butyrate addition to serum-free suspension culture of bcl-2-overexpressing CHO cells. Biotechnol Prog 21(1):50–57

Sung YH, Song YJ, Lim SW, Chung JY, Lee GM (2004) Effect of sodium butyrate on the production, heterogeneity and biological activity of human thrombopoietin by recombinant Chinese hamster ovary cells. J Biotechnol 112(3):323–335

Vanhoutvin SA, Troost FJ, Hamer HM, Lindsey PJ, Koek GH, Jonkers DM, Kodde A, Venema K, Brummer RJ (2009) Butyrate-induced transcriptional changes in human colonic mucosa. PLoS One 4(8):e6759

Vlasak J, Ionescu R (2008) Heterogeneity of monoclonal antibodies revealed by charge-sensitive methods. Curr Pharm Biotechnol 9(6):468–481

Yoon SK, Hong JK, Lee GM (2004) Effect of simultaneous application of stressful culture conditions on specific productivity and heterogeneity of erythropoietin in Chinese hamster ovary cells. Biotechnol Prog 20(4):1293–1296

Zhang T, Bourret J, Cano T (2011) Isolation and characterization of therapeutic antibody charge variants using cation exchange displacement chromatography. J Chromatogr A 1218(31):5079–5086