Stable expression of recombinant human coagulation factor XIII in protein-free suspension culture of Chinese hamster ovary cells

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Abstract

The recombinant *a* and *b* subunits for human coagulation factor XIII were transfected into Chinese hamster ovary (CHO) cells. CHO cells were amplified and selected with methotrexate in adherent cultures containing serum, and CHO 1-62 cells were later selected in protein-free medium. To develop a recombinant factor XIII production process in a suspension culture, we have investigated the growth characteristics of CHO cells and the maintenance of factor XIII expression in the culture medium. Suspension adaptation of CHO cells was performed in protein-free medium, GC-CHO-PI, by two methods, such as serum weaning and direct switching from serum containing media to protein-free media. Although the growth of CHO cells in suspension culture was affected initially by serum depletion, cell specific productivity of factor XIII showed only minor changes by the direct switching to protein-free medium during a suspension culture. As for the long-term stability of factor XIII, CHO 1-62 cells showed a stable expression of factor XIII in protein-free condition for 1000 h. These results indicate that the CHO 1-62 cells can be adapted to express recombinant human factor XIII in a stable manner in suspension culture using a protein-free medium. Our results demonstrate that enhanced cell growth in a continuous manner is achievable for factor XIII production in a protein-free medium when a perfusion bioreactor culture system with a spin filter is employed.

Introduction

Human coagulation factor XIII, known as also plasma transglutaminase or fibrin stabilizing factor, circulates in blood as a heterotetramer consisting, of two catalytic *a* and two noncatalytic *b* subunits (Schwartz et al. 1973; Ichinose et al. 1986; Bröker et al. 1991). Factor XIII is a proenzyme, which is activated by thrombin that is generated at the final stage of the blood coagulation cascade. A deficiency of factor XIII in humans results in a life-long bleeding tendency, spontaneous abortion, and abnormal wound healing. Although the secretion pathway of factor XIII is not clearly known, studies have been performed to ex-

amine the biological roles of the factor by using animal cell expression system. The *a* subunit of factor XIII displays features of a typical cytoplasmic protein, and on the other hand, the *b* subunit has a typical sequence for secretion (Ichinose et al. 1986). In a study on the release of the *a*, *b* subunits of factor XIII expressed in BHK cells, the *a* subunit was found to be released from cells only as a consequence of cell injury at least *in vitro* (Kaetsu et al. 1996).

Recently the expression of human factor XIII was achieved in several host cells, *Escherichia coli*, Chinese hamster ovary (CHO), Baby hamster kidney (BHK) and yeast (Bröker et al. 1991; Tharaud et al. 1992; Kaczmarek et al. 1995; Kaetsu et al. 1996). The

yeast cell expression system was shown to be an efficient system for the expression of recombinant factor XIII, resulting in a high yield. Although the animal cell culture system is hampered by slower cell growth, lower cell density and lower production rate than that of yeast, the expression of factor XIII in an animal cell culture system is still believed to be the method of choice as for many other human recombinant proteins for commercial production. CHO cells have been widely used for expression of human therapeutic recombinant proteins such as blood clotting factors, cytokines, growth factors, immunoglobulins, and thrombolytics (Kaufman et al. 1988; Boedeker 1992; Hayter et al. 1992; Tonouchi et al. 1992; Bekkari et al. 1994; Castro et al. 1995; Kaufman et al. 1985). The wide use of CHO cells has been due to several advantages: relative stability and high expression levels, efficient posttranscriptional processing, glycosylation of recombinant proteins, easy FDA approval for commercial production, and the scale up capacity using serum-free and protein-free medium.

In order to develop a CHO based production system for human recombinant coagulation factor XIII, CHO cells expressing the *a* and *b* subunits of recombinant factor XIII were selected in a stationary culture in serum containing medium. Up to now, the expression of factor XIII in a suspension culture using protein-free media has not been reported, and it has been difficult to get factor XIII secreted into the culture media. In addition, it is not easy to culture adherent animal cells on a large scale because of cumbersome handling, higher contamination risk and high labor cost. Thus, the development of a serum-free or protein-free culture medium is a key point for the large-scale industrial production of a target protein from animal cell culture based on the use of CHO cells, because under serum-free conditions these cells grow in suspension and also because the biological safety is increased.

In this study, we developed a protein-free medium for a CHO cell suspension culture. The adaptation of CHO cells to suspension culture was performed by weaning or direct switching to serum-free conditions. Via evaluation of long-term stability of factor XIII expression in a protein-free suspension culture, it was possible to perform a scale-up. Thus, a scale-up culture of CHO cells in a stirred tank bioreactor with a spin-filter was successfully performed by using a protein-free medium. This procedure will allow high-density cultures of CHO cells for the production of recombinant proteins.

Cell line and cell culture

The anchorage-dependent CHO cells which express recombinant human factor XIII were constructed using the following transfection strategy. The dhfr negative (dhfr⁻ host CHO cell line, DG44, was grown in Minimal essential medium alpha modification (MEM alpha, Sigma, USA) with 10% dialyzed fetal bovine serum (Sigma, USA). The CHO cells were co-transfected with 10 µg of each of the expression plasmids coding the *a* (with dhfr gene) and *b* subunits (without the dhfr gene) of factor XIII using Lipofectin® (Life Technologies, USA). The *Hind* III 5' end, containing the leader sequence, *Apa* I 13' end of the 2.3 kb coding region of the *a* subunit cDNA of factor XIII, was constructed by RT-PCR (reverse transcriptase polymerase chain reaction) from human liver RNA. The 2.2 kb coding region for the *b* subunit cDNA was constructed from human placental RNA with the same cloning site. The expression vectors for the *a* subunit and *b* subunit were named pRC-CMV (dhfr)/XIIIa and pRC-CMV/XIIIb. The construction of expression plasmids was carried out by using an established protocol (Jin et al. 1993). Both plasmids contained the neo gene as selection marker. After transfection, cells were harvested and aliquots of 100 µl were distributed in 96 well plates at a cell concentration of 1×10^4 cells per well. The cells were cultivated in the presence of 5 mg ml⁻¹ G418® (Life Technologies, USA) for the selection of transfectants. In order to obtain stable clones, a selective agent, methotrexate (MTX) was added to the each well. During the MTX selection and adaptation with increasing MTX concentration from 20 nM, 80 nM, 320 nM, to 1 µM, MTX-resistant subclones for factor XIII production were screened by an ELISA and by a modified biological activity assay. Finally, the 1-62 clone was obtained from the selection of a single cell subclone. The selected CEO 1-62 cells expressing factor XIII were maintained in MEM alpha with 5%FBS and 1 µM MTX at 37 °C under a humidified atmosphere containing 5% CO₂. Adherent cell cultures in 175-cm² T-flask (Nunc, Denmark) were subcultured twice per week by treatment with a 0.1% trypsin solution.

Protein-free media for CHO cell culture

The protein-free medium, GC-CHO-PI, was developed for suspension culture of CHO cells by the Green Cross company (Korea). The GC-CHO-PI medium formulated for optimal cell growth and pro-

tein production in CHO cells was manufactured by Sigma-Aldrich (USA) under cGMP conditions. This protein-free medium was originally designed as medium free of animal-derived components. The basal medium consisted of a modification of Dulbecco's Modified Eagle medium and Ham's F12 medium without hypoxanthine, thymidine, and phenol red. This basal medium for protein-free culture of CHO cells was supplemented with additional amino acids, vitamins, and serum-free supplements, such as lipoic acid, linoleic acid, hydrocortisone, trace elements, sodium selenite, and sodium pyruvate. The osmolality was controlled at 290 ± 10 mOsm per kg with sodium chloride. The protein-free medium was finally supplemented with 01% Pluronic F68[®] for suspension culture.

Perfusion culture system

After CHO cells expressing the *a* and *b* subunits of recombinant factor XIII were adapted for suspension growth in protein-free medium, spinner cultures (flasks from Bellco, USA) of 1-62 cells were started at an initial concentration of $3-4 \times 10^5$ cells ml⁻¹ using an agitation of 50 rpm (biological stirrer from Thermolyne, USA). All cultures were supplemented with 1 μ M MTX. After the cell density reached about 1×10^6 cells ml⁻¹, the culture medium was replaced by fresh protein-free medium at 85% of the working volume.

Perfusion cultures were performed using a 10-l working volume stirred tank bioreactor (BIOSTAT[®] ED, B.Braun Biotech Inc., Germany) equipped with a stainless steel spin filter (pore size 15 μ m) mounted on the agitation shaft. Fresh medium was dispensed into extra-spin filter space and cell-free supernatant was withdrawn from the spin filter. Seed cells were prepared in 3 l spinner flasks and inoculated into the bioreactor. A digital control unit (DCU) system was used to control the pH, agitation, dissolved oxygen and temperature. The composition of the culture medium was the same as that used in the spinner flask cultures. The perfusion was initiated after the cell density has reached about 1×10^6 cells ml⁻¹ by adjusting the pumping rate of peristaltic pumps. Dissolved oxygen (D.O.) was maintained at 40% of air saturation at the atmospheric pressure, and the pH value was maintained at 7.1 by adding CO₂ to the gas stream or by adding a NaHCO₃ solution to the medium. Samples were taken twice daily for the determination of glucose and lactate concentration and the cell density. Samples were centrifuged and the

supernatants were stored at -70 °C for further analyses.

In vitro bioassay of factor XIII

A modified factor XIII assay method was used to determine the enzyme activity from CHO cell lysates that express factor XIII. For the preparation of 1% (w/v) factor XIII free monomer solution, 4 g of fibrinectin was dissolved in 400 ml of citrate buffer (50 mM, pH 6.8), and 100 μ l thrombin was added to produce a fibrin clot. The chopped fibrin clots were washed with 100 ml of citrate buffer (50 mM, pH 6.8) until soluble protein was nonexistent by centrifugation at 3,000 g (Beckman Avanti M J-25 centrifuge, USA). After discarding the supernatant, the pellet was dissolved in 5 M urea by stirring gently. The solution was dialyzed 3 times against 2 mM acetic acid and adjusted to 20 mg ml⁻¹. The solution was then lyophilized, at which point it was ready to be used for assay by dissolving in 2 ml of 2 mM acetic acid.

The CHO cell lysates were prepared from cultured CHO cells by centrifugation at 8,000 g for 20 min. The pellets were dissolved in the 0.01 M Tris-acetate buffer (pH 8.0) and disrupted by freezing and thawing. The cell lysates were harvested by centrifugation again, and the original culture supernatants were mixed with cell lysates. After the standard factor XIII human plasma was diluted with 0.15 M NaCl/0.4% albumin solution to 0 mU ml⁻¹, 5 mU ml⁻¹, 10 mU ml⁻¹, 20 mU ml⁻¹, 25 mU ml⁻¹. 100 μ l of 1% (w/v) factor XIII free fibrin monomer solution were added to 200 μ l of each standard solution and samples in the test tubes. Thrombin was prepared by dilution of thrombin (250 U lyophilized powder) with four parts of 1 M Tris-acetate (pH 8.0) buffer solution containing 0.125 M CaCl₂ and one part of 0.15 M NaCl in the 0.4% albumin solution into final 2 ml. After incubation for 1 hr at 37 °C 3 ml of 1% (w/v) monochloroacetate solution was added to each tube. The concentrations of dissolved fibrin solution were inversely correlated with the increasing level of FXIII standard.

Other assays

During the incubation period, samples from cell culture were taken periodically for microscopic examination and for determining the cell and metabolite concentrations. Viable cells were counted by using the trypan blue dye exclusion method. Quantitative estimations of the residual glucose concentration and the

produced lactic acid concentration were carried out using an YSI Biochemical analyzer (YSI 2700, USA). The arrimonina concentration was determined by UV detection using L-glutamate dehydrogenase kit (Sigma 171-A, USA).

Results and discussion

Cell growth and Factor XIII expression in static culture

We have investigated the cell growth characteristics of recombinant CHO 1-62 cells in 175-cm² T-flasks using MEM alpha medium containing serum. CHO cells were inoculated at a cell density of 1×10^4 cells per cm² and 2×10^4 cells per cm² in medium containing different concentrations of FBS (0.5%, 1%, 2%, 3%, 4%, or 5%) and cultured in a static mode. As shown in Figure 1, cell growth rate and maximum cell concentration were increased with an increase in the serum concentrations regardless of the inoculation size, and cultures containing 4% and 5% FBS showed similar maximal cell growth (approximately 2.7×10^5 cells per cm²). However, cell growth of cultures started with 2×10^4 cells per cm² was more than two times higher than that of cultures started with 1×10^4 cells per cm². This indicates that higher inocula in cultures containing more than 3% FBS have an effect on the cell growth of CHO 1-62 cells in adherent cultures. The culture time to obtain maximum cell growth was reduced with higher inoculum and higher serum concentrations (data not shown). This result shows that recombinant CHO 1-62 cells in static culture have a typical cell growth characteristic, dependent on the inoculum size and the serum concentration. Hence, 1-62 cell growth could be optimized by adequate determination of the inoculum size and serum concentration. Adherent cultures were typically practiced in batch mode without pH control.

Recently, cell culture media manufacturers commercialized several protein-free media for CHO cells, and several pharmaceutical companies producing recombinant therapeutic proteins for human use have developed their own protein-free media. Nevertheless, there have been few reports on protein-free suspension culture media for industrial production of recombinant human therapeutic proteins from CHO cells. Although, CHO cell lines are generally cultured in adherence, the cells can also be cultured in suspension using commercialized or custom-made protein-free

Figure 1. Kinetics of cell growth at different concentrations of FBS in static cultures: profiles of cell growth of cultures started with 1×10^4 cells per cm² (a) or with 2×10^4 cells per cm², (b) are presented.

media. For suspension culture, adaptation to serum-free and suspension culture is often required. We have investigated the expression of recombinant factor XIII in a static culture of 1-62 cells by using several commercialized serum-free and protein-free media as well as the GC-CHO-PI medium. The CHO 1-62 cultures were started with a concentration of 2×10^4 cells per cm² in MEM alpha supplemented with 5% FBS. When cell cultures were above 90% confluency, the culture medium was replaced by various media, such as CHO-S-SFM II (Life Technologies, USA), HyQCCM5 (Hyclone, USA), Ex-Cell™ 301 (JRH Bioscience, USA), GC-CHO-PI, and MEM alpha with 5% FBS. The relative expression levels of factor XIII in all cultures, with exception of the culture done in Ex-Cell™ 301 medium, were a little bit higher than that in serum supplemented medium (Figure 2). This indicates that the expression of factor XIII in static cultures was not significantly affected by different culture media. Factor XII activity was barely detected

in the culture supernatants. This may be due to the characteristics of the *a* subunit maintained within adherent CHO 1-62 cells. However, the *b* subunit of factor XIII was detected in the culture supernatants. These results correspond to the study that the *a* subunit of factor XIII displays the features of a typical cytoplasmic protein whereas the *b* subunit is normally secreted into the medium (Ichinose et al. 1986).

Adaptation to protein-free suspension culture

As suspension culture systems of animal cell offer many advantages for the production of recombinant proteins, suspension of adherent cells are advantageous for the scale-up production of recombinant proteins. CHO or BHK cell lines for expression of human therapeutic recombinant proteins are anchorage-dependent, and suspension cultures of these cells using serum and serum-free media have been conducted for the purpose of industrial production of recombinant proteins. A recent study indicates that engineering the regulatory structure of the mammalian cell cycle is an alternative method for suspension culture adaptation in the absence of serum (Lee et al. 1996). The adaptation of cells to cultivation in serum-free medium is usually achieved by gradual weaning. Despite these possibilities, not all cell lines are

adopted to suspension growth in serum-free or protein-free medium.

To adapt 1-62 cells gradually to suspension culture and finally to growth in protein-free medium, suspension adaptation of these cells to protein-free media was performed using a modified weaning method and the direct switch method. CHO 1-62 cells grown in MEM alpha medium containing serum were adapted to protein-free suspension culture using a two-step procedure; the first step was a sequential reduction of the serum concentration in static culture, and the next step was the selection of CHO cells showing an elevated cell growth rate in suspension culture. During the weaning period, cultures were performed by stepwise reduction of serum from 2.5%, 1.25%, 0.5%, to 0.25% and finally to the protein-free GC-CHO-PI medium (Figure 3). During the adaptation period to suspension growth, cell aggregates of more than 10 cells were removed by precipitation. Subsequently, the cell suspension was centrifuged at 1,000 rpm for 5 min, and the pellet was resuspended with the reduced serum medium or protein-free medium. Samples of each culture were collected for the factor XIII assay. Cells were inoculated into 4–5 parallel cultures for each passage at 4×10^5 cells ml^{-1} in the GC-CHO-PI medium containing different serum concentrations, and the culture showing the highest population doubling in the suspension culture was selected and subcultured in fresh medium. Although the cell growth rate decreased with decreasing serum concentrations (from 0.031 h^{-1} to 0.024 h^{-1}) during gradual adaptation to medium with reduced serum concentrations, cell viability remained high at about 93–97%. After 15 subcultures (approximately 48 population doubling levels) the CHO 1-62 cell line was successfully adapted to growth in the protein-free medium, GC-CHO-PI. Although we had conducted similar procedures to adapt CHO 1-62 cells to other commercialized serum-free or protein-free culture media, such as CHO-S-SFM II, HyQ-CCM5 or Ex-Cell™ 325 PF-CHO, 1-62 cells failed to adapt to growth in the absence of serum (data not shown).

In general, most commercial serum-free or protein-free media cannot support cell growth after a direct switch from adherent culture in serum-containing media to suspension culture in serum-free or protein-free media. Strict requirements should be met for adaptation of adherent cells to suspension culture using serum-free or protein-free media. A CHO 1-62 cell suspension at a concentration of 6×10^5 cells ml^{-1} obtained directly from T-flasks was used as

Figure 2. Comparison of factor XIII expression in different commercial serum-free media: CHO-S-SFM II, Ex-Cell 301, GC-CHO-PI, HyQ-CCM5, and MEM with 5% FBS. The CHO 1-62 cells were inoculated at a concentration of 2×10^4 cells per cm^2 in MEM alpha medium supplemented with 5% FBS, and after 3 days of incubation the culture media were replaced by the indicated serum-free media. The reference was MEM + 5% FBS.

Figure 3. Serum weaning scheme for suspension culture of 1-62 CHO cells using the GC-CHO-PI protein-free medium. The MEM alpha medium was replaced by the protein-free medium for the 4th cell passage. Suspension culture was started at tenth passage.

inoculum for suspension cultures in spinner flasks. Before inoculation, by using the protein-free GC-CHO-PI medium the culture medium of adherent cultures was changed everyday for one week. This was done for adapting CHO 1-62 adherent cells to growth in protein-free medium without Pluronic F-68®. The cell morphology changed gradually from typical epithelial-like to a “round”. Morphology after 7 changes of the medium, the CHO 1-62 cells had a spherical shape and were slightly attached on the surface of the T-flask and spherical single cells and small aggregates were left suspended in the medium. These floating cells were then harvested by pipetting and used as inoculum for protein-free suspension culture. In each passage, suspension cultures showing an increased cell growth were selected and subcultured in the subsequent suspension culture using the protein-free medium. The selection method was carried out repeatedly during 10 passages. Initial cell concentration was decreased step by step from 6×10^5 cells ml^{-1} to 2×10^5 cells ml^{-1} . During adaptation to suspension growth in the protein-free GC-CHO-PI medium, cell growth decreased slightly with each passage, however, the viability stayed at about 91–96% (data not shown). CHO 1-62 cells grown in adherent culture did not require a weaning process to get to suspension growth in the protein-free medium. After 10 passages, CHO 1-62 cells have completely adapted to protein-free suspension culture using the GC-CHO-PI medium (Figure 4). It can be more desirable to use the high concentrations of inoculum showing higher viability to obtain stable cell growth

in suspension culture in protein-free medium. Although no significant differences in cell growth were found between adherent and suspension growth, the average growth rate of 0.027 h^{-1} (doubling time, $t_d = 25 \text{ h}$) in suspension culture was lower in comparison to the static culture containing 5% serum (0.034 h^{-1}). Using these adapted cells, the stable long-term expression of factor XIII was evaluated in protein-free suspension cultures.

Stable expression of factor XIII in suspension culture

The CHO cells adapted to serum-free condition should maintain the characteristics of importance, i.e., phenotype and protein expression. Therefore, these characteristics should be monitored in a long term cell culture process. In the case of the adapted CHO cells, productivity, gene copy number and amount of mRNA coding for the recombinant protein to be produced were maintained at the same levels as in the original cell. However, in unstable subclones, gene copy number and the amount of mRNA decreased rapidly, and reversions in the dhfr locus in CHO cells have been reported. It was also important to monitor the yield of the product for each culture in order to verify that the suspension batch did not affect the production. After adaptation of the cells to growth in protein-free suspension culture, we determined cell growth and factor XIII productivity in long-term cultures using the GC-CHO-PI medium supplemented with $1 \mu\text{M}$ MTX.

The CHO 1-62 cells, adapted to the protein-free

Figure 4. Suspension adaptation of CHO 1-62 cells to protein-free medium by direct switch from static culture to suspension. The inoculum sizes were decreased with increasing passage number.

suspension culture medium, were inoculated at a concentration of 3×10^5 cells ml^{-1} in 100-ml spinner flasks (250 ml; working volume, 100 ml) and the spinners were continuously stirred at 60 rpm. After a cell density of about 1×10^6 cells ml^{-1} was obtained, cells were harvested and new culture was initiated at a concentration of 3×10^5 cells ml^{-1} . This repeated batch mode was performed up to 20 passages (approximately 1000 h). As shown in Figure 5, growth rate was maintained at about 0.025 h^{-1} , and cell viability ranged between 90% and 97%. The factor XIII specific activity was stayed at about 14 units per 10^9 cells h^{-1} . This result indicates that growth of CHO 1-62 cells and expression of factor XIII were maintained during a long-term suspension culture using the GC-CHO-PI protein-free medium. Therefore, CHO 1-62 cells may be applied for large-scale suspension cultures.

Protein free Perfusion culture

In principle, a perfusion bioreactor retains cells inside, while conditioned medium is constantly replaced by fresh medium. The interest of the spin filter technology is its ability to retain cells and to increase the cell density in the bioreactor. In recent reports, perfirion culture systems have been used for high-density cultures of CHO cells for producing recombinant proteins in serum-free media (Searles et al. 1994; Sinacore et al. 1996; Konstantinov et al. 1996; Lee et al. 1996). In batch suspension culture using the protein-free medium GC-CHO-PI, maximal cell densities of approximately 3.4×10^6 cells ml^{-1} were obtained when the cultures were inoculated with 3×10^5 cells ml^{-1} . In order to achieve higher cell densities and

therefore a higher reactor productivity, a stirred tank bioractor equipped with a spin filter was used in this study. After comparison of spin filters with different pore sizes, a spin filter with a pore size of $15 \mu\text{m}$ was chosen for the suspension cultures of CHO 1-62 cells using the GC-CHO-PI protein-free medium.

After inoculation at a concentration of 3×10^5 cells ml^{-1} , the 10-l bioreactor was operated for 3 days in a batch mode. Perfusion was started on the third day of the cultivation. At the onset of perfusion, the cell concentration was approximately 1×10^6 cells ml^{-1} . Glucose was used as the sole nutrient control factor in the perfusion culture. The residual glucose concentration was controlled at 35 mM, which equals to approximately 70% of its concentration in a fresh medium, and the lactate produced was maintained at 7–8 mM. In general, other authors (Hayter et al. 1992; Linz et al. 1997) had set the residual glucose concentration in fed-batch or perfusion cultures at much lower levels in order to reduce the production of lactate, which is often considered as an inhibiting factor for cell growth in animal cell culture. However, by maintaining high glucose concentration, we could successfully control the lactic acid concentration below 10 mM, which is not a limiting concentration for cell growth (results not shown). Using the perfusion rate chosen no amino acid limitations were observed (data not shown). Figure 6 presents the results from the 10-l perfusion run, which was operated over two weeks using protein-free medium. Approximately 200 l of culture filtrates were harvested from the 10-l bioreactor cultured in perfusion mode. Cell density kept increasing as perfusion brought in fresh medium and carried away the spent medium. After 1 week of culture, cells formed a number of small sized aggregates. As time went on, the size of cell-aggregates enlarged to reach 10–20 cells, with large aggregates containing up to 50 cells. As shown in Figure 6, CHO 1-62 cells were able to reach a maximum cell density of more than 1.7×10^7 cells ml^{-1} , which is an approximately 5-fold increase in comparison to the batch culture. Glucose and lactate levels were monitored throughout the culture as indicators for perfusion rate adjustment. Although the expression level of factor XIII secreted to culture supernatant was low with respect to the high cell density, factor XIII expression was maintained at around 0.5 unit ml^{-1} of culture supernatant during the perfusion operation (Figure 7). This indicates that recombinant factor XIII can be released successfully into the culture supernatant in a perfusion culture

Figure 5. Long-term suspension culture of CHO 1-62 cells in spinner flask using the protein-free GC-CHO-PI medium.

Figure 6. Perfusion culture of CHO 1-62 cells in a 10-l spin-filter bioreactor using protein-free medium: a, profiles of cell growth and viability; b, profiles of residual glucose and produced lactate and ammonia; c, profiles of stirring speed, pH of medium, and perfusion rate.

Figure 7. Production profile of recombinant factor XIII in protein-free perfusion culture.

system. The reason for the rather low titer is that the α subunit of factor XIII is only released from dying cells. Although cell viability was maintained at above 90% during the perfusion culture, the number of dead increased with culture time, and after 256 h of culture the concentration of dead cells stayed stable at $0.8\text{--}1.6 \times 10^6$ cells ml⁻¹. These results correspond to the release data of factor XIII in BHK cell culture (Kaetsu et al. 1996), in which a factor XIII activity of 1.5–2.5 units ml⁻¹ from lysed cells was detected.

Conclusions

The CHO 1-62 cells were selected for the expression of recombinant human coagulation factor XIII in adherent cultures containing serum. To develop a factor XIII production process using a suspension culture system, we have investigated the growth and factor XIII expression characteristics of CHO cells for adaptation to protein-free suspension culture. CHO 1-62 cells did not require weaning steps for adaptation to suspension growth in the GC-CHO-PI protein-free medium when adapting adherent cells to the protein-free medium. The cell specific productivity of factor XIII underwent only minor changes by direct adaptation to protein-free medium. The GC-CHO-PI protein-free medium was adequate for suspension culture of CHO 1-62 cells and expression of factor XIII. The 1-62 cells showed a stable expression of factor XIII under protein-free suspension culture conditions for about 1000 h. This indicates that the protein-free GC-CHO-PI medium can be used for the long-term suspension cultures of CHO cells for the production of factor XIII. The cells could be subcultured serially in the protein-free medium and achieve the same cell growth, indicating true adaptation of the cells. In a large-scale suspension culture using a spin-filter perfusion culture system, the α subunit of factor XIII was released from cells as a consequence of increased cell death. The ability of the recombinant CHO 1-62 cells to multiply in protein-free culture greatly facilitated the scale-up of the perfusion culture. The efficient expression of factor XIII in the perfusion culture will be further studied by controlling cell viability and by supplementing with secretion factors.

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