Yeast hydrolysate as a low-cost additive to serum-free medium for the production of human thrombopoietin in suspension cultures of Chinese hamster ovary cells

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Abstract

To enhance the performance of a serum-free medium (SFM) for human thrombopoietin (hTPO) production in suspension cultures of recombinant Chinese hamster ovary (rCHO) cells, several low-cost hydrolysates such as yeast hydrolysate (YH), soy hydrolysate, wheat gluten hydrolysate and rice hydrolysate were tested as medium additives. Among various hydrolysates tested, the positive effect of YH on hTPO production was most significant. When 5 g/L YH was added to SFM, the maximum hTPO concentration in batch culture was 40.41 μg/mL, which is 11.5 times higher than that in SFM without YH supplementation. This enhanced hTPO production in YH-supplemented SFM was obtained by the combined effect of enhanced q_{hTPO} and increased culture longevity. In addition, YH supplementation did not affect in vivo biological activity of hTPO. Taken together, the results obtained demonstrate the potential of YH as a medium additive for hTPO production in serum-free suspension cultures of rCHO cells.

INTRODUCTION

Human thrombopoietin (hTPO) is a potential therapeutic glycoprotein for the amelioration of thrombocytopenia associated with chemotherapy, irradiation and bone marrow transplantation[1]. The hTPO has been expressed in Chinese hamster ovary (CHO) cells, but its expression level is low (0.7 - 8.5 μg/mL)[2]. To increase the hTPO expression level, culture medium specifically designed for hTPO production needs to be developed.

In the present study, we assessed the efficacy of several non-animal derived hydrolysates as additives to SFM for high-level expression of hTPO in suspension culture of recombinant CHO (rCHO) cells.

MATERIALS AND METHODS

The rCHO cells producing hTPO (R-2-3-2) were used in this study. Cells were cultured as suspension cultures in 125 mL Erlenmeyer flasks (Corning, Corning, NY) containing 50 mL of medium on an orbital shaker (Vision, Bucheon, Korea) at 135 rpm in a 5% CO_2/air mixture, humidified at 37℃. hTPO concentrations were quantified using an enzyme linked immunosorbent assay (ELISA). hTPO mRNA was characterized by Northern blot hybridization with the hTPO probe. The in vivo biological activities of hTPO produced in various serum-free media were tested in mice.

RESULTS AND DISCUSSION

Cell Growth and hTPO Production

Figure 1 shows typical profiles of cell growth and hTPO production during batch cultures. Among the hydrolysates tested, the supplementation of YH in SFM was most beneficial to hTPO production, resulting in more than 11-fold increase in the maximum hTPO concentration. This result was obtained by the combined effect of enhanced q_{hTPO} and extended culture longevity. The supplementation of YH in SFM increased q_{hTPO} by 294% and extended culture longevity by 2 days if the culture was terminated at a cell viability of 50%. Furthermore, cell viability during the culture using YH-supplemented SFM was higher than that using any other medium tested and thereby cell lysis liberating proteases and glycosidases from dead cells was least significant. Thus, degradation of hTPO susceptible to proteolytic and/or intrinsic degradation could be minimized in YH-supplemented SFM.

hTPO mRNA and Intracellular hTPO Level

To understand the hydrolysate effect on q_{hTPO} at the transcription level, the relative hTPO mRNA contents were quantified using Northern blot analysis.

Figure 2 shows the Northern blots of hTPO and β-actin mRNAs prepared from cell in mid-exponential growth phase during the cultures shown in Fig. 1A. Like q_{hTPO}, the relative hTPO mRNA content from cells in YH-supplemented SFM was higher than that in any other medium, suggesting that the increased transcription level of hTPO was responsible in part for the enhanced q_{hTPO} in YH-supplemented medium. Like hTPO mRNA level, the intracellular hTPO content per cell in YH-supplemented SFM was also

higher than that in any other medium.

In Vivo Biological Activity

Given the fact that hTPO is a molecule that is heavily dependent on the cells ability to make posttranslational modifications, supplementation of YH may also affect in vivo biological activity of hTPO.

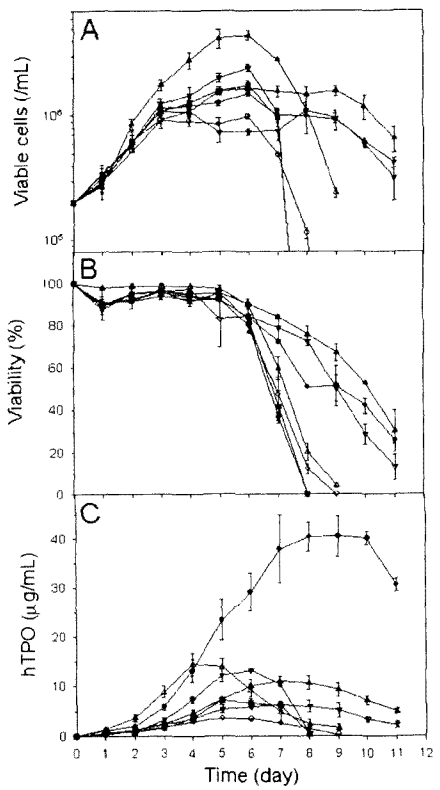

Figure 1. Batch cultures using various SFM supplemented with 5 g/L of corresponding hydrolysate. **A:** Cell growth. **B:** cell viability. **C:** hTPO production. (O): SFM; (●): SFM supplemented with YH; (▲): SFM supplemented with SH; (▼) SFM supplemented with WGH; (■): SFM supplemented with RH; (△): IMDM supplemented with 10% dFBS; (▽): CHO-S-SFM II. Error bars represent the standard deviation determined in duplicate experiments.

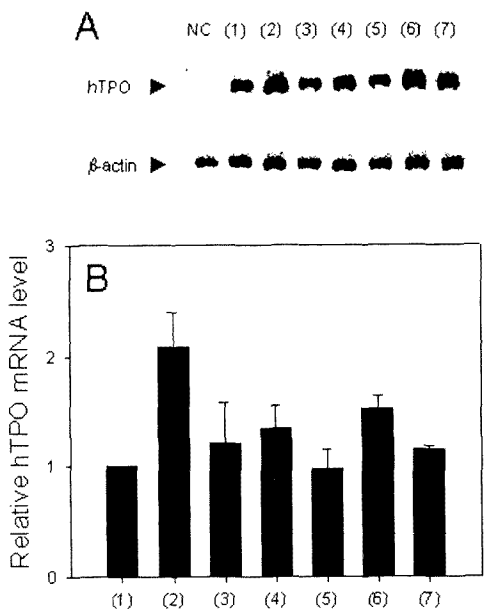

Figure 2. Northern blot analysis of hTPO gene expression in SFM supplemented with corresponding hydrolysate. **A:** Northern blot. **B:** Relative hTPO mRNA level. The ratio hTPO/β-actin shows the relative level of hTPO mRNA normalized with the internal control, b-actin mRNA. The cytoplasmic RNA of DUKX-B11 was used as a negative control (NC). (1): SFM; (2): SFM supplemented with YH; (3): SFM supplemented with SH; (4): SFM supplemented with WGH; (5): SFM supplemented with RH; (6): IMDM supplemented with 10% dFBS; (7): CHO-S-SFM II.

Figure 3 shows the changes in platelet levels of mice after a single injection of hTPO samples at a concentration of 20 μg/kg. The biological activity of hTPO produced in YH-supplemented SFM was similar to that in SFM and CHO-S-SFM II, but was significantly higher than that of negative control, as shown by statistical analysis using t-test ($P<0.05$, n=4-6). This result implies that YH supplementation enhances q_{hTPO} of rCHO cells without deteriorating the quality of hTPO.

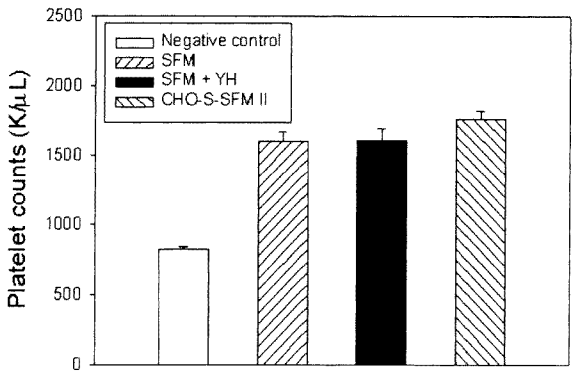

Figure 3. Effect of YH- supplementation to SFM on the in vivo biological activity of hTPO. Error bars represent the standard deviation determined in 4-6 animals.

CONCLUSION

The addition of YH (5 g/L) to SFM significantly increased the maximum hTPO concentration in suspension culture of rCHO cells as a result of enhanced q_{hTPO} and extended culture longevity. Further, it did not affect in vivo biological activity of hTPO, demonstrating the potential of YH as medium additives.

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